Unravelling the reproductive tactics of a tropical clupeid fish (*Ethmalosa fimbriata*, Bowdich 1825) against the backdrop of climate change

Julian Döring

Unravelling the reproductive tactics of a tropical clupeid fish (*Ethmalosa fimbriata*, Bowdich 1825) against the backdrop of climate change

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Julian Döring

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1st Examiner

Dr. Werner Ekau, Leibniz Centre for Tropical Marine Research (ZMT), Bremen

2nd Examiner

Prof. Dr. Wilhelm Hagen, University of Bremen, Bremen

Chairman of the PhD Examination Committee

Prof. Dr. Martin Zimmer, University of Bremen, Leibniz Centre for Tropical Marine Research (ZMT), Bremen

Additional Thesis Committee Members

Dr. Holger Haslob, Thünen Institute of Sea Fisheries (TI), Hamburg

Inken Hanke, PhD candidate, Leibniz Centre for Tropical Marine Research (ZMT), Bremen

Sarah I. Neumann, Master student, University Bremen

Date of PhD Colloquium

29th May 2018


AWA

Complementary Soundtrack

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SUMMARY

Clupeid fishes employ specialized reproductive tactics in response to hydrographic fluctuations in their spawning habitats. Unprecedented environmental conditions induced by climate change will challenge these behavioural and physiological adaptations. In the case of maladaptation coupled with no genetic evolution, shifts in clupeid distribution will be undermined and changes in stock sizes are foreseeable. Taking into account the key drivers of stock productivity, this thesis aimed at assessing the reproductive adaptations of a clupeid fish species, *Ethmalosa fimbriata*, towards hydrographic alterations in its spawning habitats: the upwelling area at the Senegalese South Coast and the inverse hypersaline Sine Saloum estuary. Monthly sampling of mature females was carried out from February to October 2014, during the peak of the species' extended spawning season.

In teleost fishes, little is known about the concerted influence of temperature and salinity on an individual female's condition, growth, and consequently on the amount of eggs it may produce. In **Chapter I**, the applied multiple linear regression models on the basis of *E. fimbriata*'s batch fecundity and gonadal energy storage show that clupeids can adapt towards increasing their reproductive effort at water temperatures (26 - 30 °C) and salinities (42 - 51), which by far exceed marine conditions, in an effort to maximize recruitment success. This spawning tactic, however, was accompanied by a trade-off between reproductive investment and somatic growth, which will ultimately limit the species' reproductive potential inside the hypersaline estuary.

Still, hatching success and survival probabilities of marine fish eggs and early life stages are prone to impairment under the temperature and salinity regimes encountered inside the estuary. Essential fatty acids affect osmoregulation in marine fish early life stages, and deficiencies can therefore be expected to cause elevated offspring mortality under hypersaline conditions, ultimately modifying a stock's reproductive potential. As described in **Chapter II**, oocytes spawned by *E. fimbriata* inside the hypersaline estuary exhibited significantly higher 20:4 (n-6) proportions (1.6 ± 0.7% of total fatty acids) than oocytes spawned under marine conditions (0.6 ± 0.2%). Further, oocyte proportions in 20:4 (n-6), 20:5 (n-3), and 22:6 (n-3) were positively correlated with water temperature, salinity, and female reproductive investment. Oocytes spawned during high temperature/high salinity conditions inside the estuary are therefore likely to develop normally under the prevailing conditions. Reproductive potential was determined to be highest in females spawning inside the estuary at the end of the wet season, when temperatures are high and salinities are less stressful due to freshwater input.

The entire stock's productivity is subject to variations in reproductive potential of individual females, which in turn is subject to the surplus energy available for spawning. I will show in **Chapter III** that females spawning in the estuary's middle reaches invested almost three times more energy into reproduction (115 ± 65 J g^{-1} spawner) than their neritic counterparts (39 ± 34 J g^{-1} spawner). In order to identify spawning areas and to discriminate among stock components, elemental analysis of fish otoliths is a widely used methodology. Female otolith levels in Ba:Ca, Sr:Ca, and Zn:Ca either differed significantly between study sites or could be linked to heterogeneous environmental parameters. A linear discriminant analysis combining spawning energetics and otolith microchemistry yielded high classification percentages and thus evidenced distinct stock spawning components of *E. fimbriata* in southern Senegalese waters. These components are characterized by delimited home ranges and distinctive productivity.

E. fimbriata is so far likely to benefit from the severe impacts of climate change on its spawning habitat because of a complex stock structure and by employing auspicious reproductive tactics. The adaptations described herein potentially allow for outcompeting other pelagic fish species with lower adaptive potential. All in all, high plasticity in reproductive traits combined with high fecundities and small generation times in clupeid fishes such as *E. fimbriata* may lead to an enhanced fitness under rapid environmental changes. An ecosystem approach to fisheries should not only take into account the impact of fisheries on the ecosystem but also the ecosystem's influence on fisheries. The ecosystem impacts a fishery in several ways, among others, modifying the reproductive potential of target species. Thus, any factor altering species' habitat characteristics has concomitant effects on reproductive success in clupeids. With this in mind, a further decrease in precipitation and a concerted elevation of ambient salinities in the Sine Saloum estuary will have negative impacts on *E. fimbriata*'s reproductive potential, on the carrying capacity of its habitat, and ultimately on its stock size. Given the species high adaptability, poleward expansion of its distribution range in future temperature regimes seems probable.

ZUSAMMENFASSUNG

Heringe reagieren mit spezialisierten Fortpflanzungstaktiken auf hydrografische Veränderungen in ihren Laichhabitaten. Noch nie dagewesene, durch den Klimawandel hervorgerufene Umweltbedingungen werden diese Verhaltens- und physiologischen Anpassungen herausfordern. Im Falle von Fehlanpassungen, gekoppelt mit mangelnder genetischer Evolution, würden Verschiebungen in den geografischen Verteilungen von Heringen verhindert werden. Veränderungen der Bestandsgrößen wären somit vorhersehbar. Unter Berücksichtigung der Schlüsselfaktoren für die Produktivität von Fischpopulationen wurden in dieser Arbeit die Reproduktionsanpassungen einer Heringsart, *Ethmalosa fimbriata*, an hydrographischen Veränderungen in ihren Laichhabitaten untersucht. Diese Habitate beinhalteten das Auftriebsgebiet an der senegalesischen Südküste und das inverse, hochsalzhaltige Ästuar Sine Saloum. Im Zuge dessen wurden laichbereite Weibchen während ihrer Laichzeit von Februar bis Oktober 2014 beprobt.

In Knochenfischen ist wenig über den konzertierten Einfluss von Temperatur und Salzgehalt auf die somatische Kondition sowie auf das Wachstum von Weibchen und folglich, auf die Menge der Eier, die diese produzieren können (ihre Fruchtbarkeit), bekannt. In **Kapitel I** zeigen Regressionsmodelle basierend auf dem gonadalen Energiespeicher und der Fruchtbarkeit *E. fimbriatas*, dass Heringe bei Wassertemperaturen (26 - 30°C) und Salzgehalten (42 - 51), welche marine Bedingungen bei weitem übertreffen, ihre Reproduktionsleistung steigern können. Weibchen sind somit dahingehend angepasst, dass sie ihren Rekrutierungserfolg unter hochsalzigen Bedingungen durch erhöhte Fruchtbarkeit zu maximieren versuchen. Die beobachtete Laichtaktik ging jedoch mit einem physiologischen Kompromiss zwischen reproduktiver Investition und somatischem Wachstum einher. Dieser Kompromiss begrenzt letztlich *E. fimbriatas* Fortpflanzungspotenzial innerhalb des hochsalzigen Ästuars.

In Meeresfischen muss davon ausgegangen werden, dass die Überlebenswahrscheinlichkeiten der unter hochsalzhaltigen Bedingungen gelaichten Eier vermindert sind. Essentielle Fettsäuren beeinflussen die Osmoregulation in frühen Lebensstadien von Fischen und Mängel könnten eine erhöhte Sterblichkeit der Nachkommen verursachen, was letztlich das Reproduktionspotenzial des gesamten Fischbestands verändert. In **Kapitel II** wird beschrieben, dass Oozyten von *E. fimbriata*, welche innerhalb des Ästuars produziert wurden, signifikant höhere Anteile der essentiellen Fettsäure 20:4 (n-6) (1,6 ± 0,7% der Gesamtfettsäuren) aufweisen, als Oozyten, die unter marinen Bedingungen erzeugt wurden (0,6 ± 0,2%). Außerdem korrelierten die Oozytenanteile von 20:4 (n-6), 20:5 (n-3) und 22:6 (n-3) positiv mit der Wassertemperatur, dem Salzgehalt und der weiblichen

Reproduktionsinvestition. Oozyten, die bei hohen Wassertemperaturen und hohen Salzgehalten innerhalb des Ästuars gelaicht werden, entwickeln sich daher wahrscheinlich normal unter den vorherrschenden Bedingungen. Das Reproduktionspotenzial kann als am höchsten in denjenigen Weibchen bestimmt werden, welche am Ende der Regenzeit laichen. Zu diesem Zeitpunkt sind die Wassertemperaturen zwar am höchsten, dennoch nimmt der Salzgehalt aufgrund von Süßwassereintrag wieder ab, was somit auch den Umweltstress auf die Eier reduziert.

Die Produktivität eines Fischbestandes unterliegt Schwankungen im Reproduktionspotenzial einzelner Weibchen, welches wiederum der überschüssigen Energie unterliegt, die für das Laichen zur Verfügung steht. Ich zeige in **Kapitel III**, dass im Mittellauf laichende Weibchen fast dreimal mehr Energie in die Fortpflanzung investierten (115 ± 65 J g^{-1} Weibchen) als ihre neritischen Gegenstücke (39 ± 34 J g^{-1} Weibchen). Um Laichgebiete zu identifizieren und zwischen Bestandskomponenten zu unterscheiden, ist die Elementanalyse von Fischotolithen eine weit verbreitete Methode. Konzentrationen von Ba:Ca, Sr:Ca und Zn:Ca in den Otolithen von laichbereiten Weibchen unterschieden sich entweder signifikant zwischen den Untersuchungsstellen oder konnten mit heterogenen Umweltparametern in Verbindung gebracht werden. Eine lineare Diskriminanzanalyse, die den energetischen Laichaufwand der Weibchen mit ihrer Otolithenmikrochemie kombinierte, ergab hohe Klassifikationsprozente und wies somit unterschiedliche Bestandskomponenten von *E. fimbriata* in südlichen senegalesischen Gewässern nach. Diese Komponenten unterscheiden sich sowohl in ihrem Aktionsraum als auch in ihrer Produktivität.

Bis jetzt dürfte *E. fimbriata* aufgrund einer komplexen Bestandsstruktur und der Anwendung viel versprechender Fortpflanzungstaktiken von den schwerwiegenden Auswirkungen des Klimawandels auf ihre Laichgewässer profitieren. Möglicherweise können aufgrund der beschriebenen Anpassungen andere pelagische Fischarten, welche geringere adaptive Potenziale aufweisen, auskonkurriert werden. Alles in allem kann eine hohe Plastizität der Fortpflanzungsmerkmale in Verbindung mit hohen Fruchtbarkeiten und kleinen Generationszeiten in Heringen zu einer verbesserten Fitness bei schnellen Umweltveränderungen führen. Ein Ökosystemansatz für die Fischerei sollte nicht nur die Auswirkungen der Fischerei auf das Ökosystem berücksichtigen, sondern auch den Einfluss des Ökosystems auf die Fischerei. Das Ökosystem wirkt sich in verschiedenster Art und Weise auf eine Fischerei aus, unter anderem durch eine Modifizierung des Reproduktionspotenzials der Zielarten. Veränderungen von Habitatcharakteristika haben somit unmittelbare Auswirkungen auf den Fortpflanzungserfolg von Heringen. Vor diesem Hintergrund werden ein weiterer Rückgang der Niederschläge und eine konzertierte Erhöhung der Salinität im Sine Saloum negative Auswirkungen auf die Habitatgröße, das Fortpflanzungspotenzial und schließlich auf die Bestandsgröße von *E. fimbriata* haben. Angesichts der hohen Anpassungsfähigkeit der Art scheint eine Pol gewandte Ausdehnung ihres Verbreitungsgebiets in zukünftigen Temperaturregimen wahrscheinlich.

1. INTRODUCTION AND SCIENTIFIC BACKGROUND

1.1. Recruitment

In fishes, recruitment occurs when early life stages survive and subsequently juveniles are to be added to a fished population or mature to reproduce. Recruitment is therefore considered as the key mechanism responsible for maintaining sustainable populations. Recruitment variability, however, is a major source of fluctuations in species abundance since it is directly linked to maternal effects and characteristics of early life history stages (Pepin & Myers 1991). In the marine realm, recruitment is accomplished in various ways; some fish species spawn eggs, others larvae, and a few even give birth to juveniles. Within each of these groups there are several different reproductive strategies and tactics. From a fisheries management perspective the investigation of recruitment processes is of importance to comprehend the underlying effects controlling variability in marine fish populations.

Efforts to quantify recruitment provide unique opportunities to study processes regulating fish populations, thereby understanding and predicting the impacts of a changing climate and exploitation on living marine resources (Jakobsen et al. 2009). Main drivers for successful recruitment include populations' diversity in spatiotemporal reproductive behaviour, an increasing reproductive value with fish age/length, spawning stock biomass (SSB), and stock structuring (Trippel 1999, Frank & Brickman 2001, Berkeley et al. 2004, Hixon et al. 2014, Lowerre-Barbieri et al. 2017). Several studies have shown that SSB renders inefficient to predict recruitment in marine fishes (Marshall et al. 1998, Frank & Brickman 2001, Lambert 2008). It thus becomes increasingly evident that more sensitive measures for a stock's capacity to reproduce itself (i.e. its reproductive potential) are needed, which also consider the encountered environmental settings (Marshall et al. 2000). Further, greater emphasis should be given on unravelling complex stock structures to determine a stock's reproductive potential in more detail and to establish more effective stock management approaches (Frank & Brickman 2001, Marshall et al. 2003).

Taking into account the key drivers of stock productivity, this thesis will aim at assessing the reproductive adaptations of a clupeid fish towards climatic alterations in its spawning habitats: the upwelling area at the Senegalese South Coast and the inverse Sine Saloum estuary. The obtained results will ultimately foster the comprehension of recruitment processes in clupeid fish stocks in tropical habitats affected by climate change.

1.2. The Stock Concept

The term "stock" refers to a population that is reproductively self-sustaining and comprises individual fish displaying identical life history traits as a response to environmental parameters (Ihssen et al. 1981). Since certain traits may be expressed differently in the same genotype, as an adaptive response to environmental factors, stock discrimination solely on the basis of genetics renders insufficient (MacLean & Evans 1981, Begg et al. 1999, Swain & Foote 1999). In fisheries management, special emphasis is given on the appraisal of stock reproductive potential since the stock's contribution to an entire fishery may vary temporally (Waldman & Fabrizio 1994). Information on survival probabilities of early life-history stages in stocks allows for predicting year-class strength and recruitment (Begg & Marteinsdottir 2000). Further, in order to understand mechanisms responsible for population structuring and recruitment variability, information on spawning location and seasonality as well as on origin of early life-history stages are needed (Marteinsdottir et al. 2000, Jakobsen et al. 2009).

Conversely, information on stock structure constitutes an essential asset to foster understanding of reproductive patterns. Exploited small pelagic fish stocks are often composed of different spawning components, and each component's contribution to the fished stock varies with its productivity (Begg et al. 1999). Generally, less productive components are more vulnerable to fishing pressure than more productive ones (Jennings et al. 1998). Thus, less productive components are more likely to be overexploited, which ultimately leads to a loss of genetic variability (Stephenson 1999). To facilitate stock assessment accuracy and to improve fisheries management plans, the relative contribution of a spawning component to the entire stock needs to be estimated. Recruitment and reproductive potential need to be determined for each component separately to avoid incorrect estimates of stock size and productivity (Hilborn 1985). Assessment of stock structures may, therefore, serve to conserve reproductive potential, and in turn, scales of management should be in accordance with those encountered in nature (Jakobsen et al. 2009).

1.3. Reproductive Modes, Strategies, and Tactics

Fishes exhibit a large range in sexuality like sequential or simultaneous hermaphroditism, unisexuality (parthenogenesis), bisexuality (gonochorism), or a combination of these (Wootton 1998, Jalabert 2005, Blaxter 2010). They express most reproductive modes known in vertebrates. These modes include: ovuliparity, oviparity, ovo-viviparity and histotrophic viviparity (Lodé 2012). Fishes have, in addition, the widest suite of reproductive strategies observed in vertebrates (Green 2008), such as parental care or parental ignorance, semelparity and iteroparity. Parental care, or investment into offspring after fertilization, ranges from simple burial of eggs to internal gestation and live-

bearing. It may involve only the female, only the male, or both sexes working concertedly (Gross & Sargent 1985). Matrotrophy, for example, describes an adaptation that facilitates the active transfer of nutrients from mother to developing offspring during gestation (Wourms 1981, Morrison et al. 2017). Other forms of parental care involve behavioural adaptations such as the guarding of demersal eggs or mouth-breeding (Oppenheimer 1970, Balon 1975). In most commercially important small pelagic fish (e.g. sardinellas, sardines, anchovies, and shads), however, there is no parental care. The eggs are untended and the gametes are released directly into the water column where they are fertilized (Barbieri et al. 2017). Semelparous organisms breed once after reaching maturity and subsequently die without significant post-reproductive survival. In semelparous fish, the physiological alterations associated with maturation and reproduction result in ramifications that inevitably lead to death (Wootton 1998). Iteroparity, on the other hand, is the case in which, after reproduction, the individual will probably survive to reproduce again (Smith & Wootton 2016). In batch spawning species, the female spawns eggs in batches (or clutches) at intervals during the reproductive season. The time between spawning events varies. Some species may spawn daily whereas others reproduce in intervals of several days. Batch spawners can be split into two categories: If females have all the eggs that are going to be spawned during that breeding season already present in their ovaries at the start of the breeding season, these spawners exhibit determined fecundity. If females are able to replenish the pool of eggs that can be spawned during the reproductive season, they are regarded as indeterminate spawners (Wootton 1998, Smith & Wootton 2016, Ganias & Lowerre-Barbieri 2018). Iteroparity and batch spawning overcome the physical constraint the size of the body cavity poses on egg production (Kamler 2005). Also, this strategy promotes bet-hedging, a valuable adaptation to unpredictable environments (Furness et al. 2015, McBride et al. 2015). Iteroparity and batch spawning also reduce mortality caused by very high, short-term levels of reproductive effort (Stearns 1992), and enhance resilience to high-mortality events in offspring (Cushing 1990, Mertz & Myers 1994). Within these species-specific reproductive strategies, fish populations adopt differentiated tactics to fine-tune reproduction. The overall goal is to ensure reproductive success, i.e. to produce offspring that will survive in a certain environment, recruit to the next generation, and is, in turn, able to reproduce (Wootton 1998, Murua & Saborido-Rey 2003).

Oogenesis is the key process that inevitably regulates reproductive strategies. It begins with the mitotic expansion of reproductive cells (oogonia), continues with the meiotic development of these cells, and ends in oocyte maturation and ovulation (Grier et al. 2009, Lubzens et al. 2010). The transition from primary to secondary oocytes is a crucial step during gonadal maturation. The production of secondary oocytes, which are to be spawned in the upcoming reproductive season, is hormone (gonadotropin) dependent (Wallace & Selman 1981, Grier et al. 2009). The initial provisioning of secondary oocytes with yolk (vitellogenesis) may, therefore, be used as an indicator to determine size and age at maturity (McBride et al. 2013). Nucleus migration is also under

hormonal control and signifies the development of tertiary oocytes (McBride et al. 2002). The process initiates oocyte maturation, is linked to the hydration of the oocytes, and ends with ovulation (McBride et al. 2015). Hydrated oocytes are thus considered as a useful indicator for estimating spawning periods and seasonality, and serve to estimate fecundity in batch spawning fishes (Murua et al. 2003, Grier et al. 2009, McBride et al. 2015). The recruitment of secondary oocytes is known to occur in three general patterns: synchronous, group synchronous, and asynchronous. In semelparous fish, a single cohort of oocytes transitions from primary to tertiary status during a fish's lifetime, a pattern referred to as "synchronous". In most iteroparous fishes, a pool of primary oocytes prevails from year to year, from which secondary oocytes develop and mature only seasonally. When this discrete cohort of secondary oocytes advances, the transition from primary to secondary oocytes is halted. This model is called "group synchronous". When cohorts of overlapping sizes of primary and secondary oocytes persist throughout the spawning season, this model is called "asynchronous" (Ganias & Lowerre-Barbieri 2018). Asynchronous ovarian development is the model predominately exhibited by small pelagic clupeoid fishes (Olney et al. 2001, Aprahamian et al. 2003, Murua & Saborido-Rey 2003, Kraus & Köster 2004). In summary, the basic sequence of oogenesis, in which the timing and rate of oocyte cohorts vary, shaped a wide range of reproductive traits, including size and age at maturity, reproductive periodicity, spawning frequency and the number of eggs produced per spawning event (McBride et al. 2015).

While the reproductive strategy of a species encompasses the various phenotypic expressions of a reproductive trait (e.g. spawning behaviour, fidelity, seasonality and frequency, size and age at first maturity, fecundity, and egg size) over the full range of environmental settings, the term "reproductive tactic" describes to which extent specific reproductive traits are expressed in a specific environmental situation (Wootton 1998, Green 2008, Jakobsen et al. 2009). Variations in the expression of these reproductive traits are most commonly adaptive responses to changing environmental parameters (Jakobsen et al. 2009, Jatteau et al. 2017). Individuals may adapt to environmental changes by altering their spawning behaviours. The parental choice of spawning site, for example, can be fundamental for offspring survival (Norcross & Shaw 1984). Egg and larval transport pathways from spawning sites to suitable retention/nursery areas are regarded as critical for recruitment (Hjort 1926, Paris et al. 2005). This is because in marine ecosystems the egg stage duration and survival of early larval stages are closely linked to physical processes, water temperatures, and food availability, all of which ultimately alter recruitment (Butler et al. 1993). Whereas tropical fishes often exhibit prolonged spawning seasons, spawning periods in temperate regions are most commonly restricted. Here, the window with favorable environmental conditions for early life stage survival is typically narrower (Jakobsen et al. 2009). Oocyte recruitment and spawning seasonality is, therefore, controlled by "ultimate factors" (e.g. endogenous rhythms and energetic thresholds) and by "proximate factors", exogenous cues (e.g. photoperiod, lunar periodicity, water temperature, and rainfall), which initiate gonadal development and

spawning activity (Ganias et al. 2015, Ganias & Lowerre-Barbieri 2018). These exogenous cues serve to predict future environmental conditions and ensure that gonadal development sets in at a suitable time so that fish are capable of spawning when conditions for offspring survival are favorable (Johannes 1978, Lowerre-Barbieri et al. 2011). Pelagic fishes such as clupeids are adapted towards spawning under optimal environmental conditions which may be accessible only for a short time, at temperatures favourable for development in early life stages, and/or at the right place/right time to ensure feeding success in larvae (Cury & Roy 1989, Cushing 1990, Roy et al. 1992, Cury & Pauly 2000).

In all scenarios energetic constraints play a crucial role in altering reproductive traits, and shaping, thereby, reproductive tactics (McBride et al. 2015). During a fish's lifespan, total available energy is budgeted towards basic maintenance, somatic growth, storage, and reproduction (Bunnell & Marschall 2003, Jørgensen et al. 2006). In the course of early life history, an individual's surplus energy is primarily allocated to somatic growth to ensure fast growth rates in an effort to reduce size-related mortality (Houde 1989, Sogard 1997, Gislason et al. 2010). Allocation of surplus energy towards somatic growth and not towards reproduction can significantly delay an individual's rate of maturation and gonad development (Marshall & Browman 2007). In the mature female, surplus energy is invested into egg batches, as well as into accessory reproductive activities such as spawning migrations, mating, and parental care (Wootton 1998, McBride et al. 2015). Prey availability and food intake also affect the amount of energy available for allocation into the gonads. Iteroparous fishes like clupeids are forced to budget between somatic maintenance and reproductive effort, potentially through periods with limited food availability, to survive and reproduce successfully (Wiegand et al. 2007).

1.4. Reproductive Potential

A fish's reproductive success is modulated by two main drivers: reproductive potential and recruitment (Lambert 2008). Early efforts in fisheries management, therefore, focused on understanding the relationship between SSB (as a proxy of reproductive potential) and availability to the fisheries (as a proxy of offspring survival to maturation). One of the prerequisites of past models integrating SSB is that many small, young, and mature females exhibit the same reproductive capacity as fewer, large, and old females of the same total mass (Jakobsen et al. 2009). This conception, however, opposes a currently widely excepted paradigm that older, and thus bigger females produce more offspring of higher quality (BOFFFF: big old fat fecund female fish) (Berkeley et al. 2004, Hixon et al. 2014). Also, the concept assumes that egg production per unit of biomass is temporally constant. Fecundity, however, may vary seasonally in relation to parental wealth (e.g. weight and condition), food availability (e.g. abundance and quality), environmental (e.g. temperature and salinity) and evolutionary stressors (e.g. stock density and

fishing pressure) (Lambert 2008). These are just two examples of why SSB is regarded as no efficient measure to predict recruitment variability in marine fish populations (Marshall et al. 2000).

Nowadays, recruitment was found to be positively related to fecundity variations and the number of eggs spawned by a stock (total egg production) thus constitutes a more reliable proxy for reproductive potential (Rickman et al. 2000). Stock-recruitment models reconstructing relationships between the size of the adult population and the number of produced offspring are therefore commonly used to assess stock viability (Marshall et al. 2006, Lambert 2008). In recent years, it became increasingly evident that a stock's reproductive potential cannot solely be assessed via the ascertainment of the number of produced gametes (Marshall et al. 2000, Jakobsen et al. 2009). To gain deeper insights into spawner-recruit relationships the physiological strength of the reproductive products must be determined in the light of the experienced environmental stressors (Scott et al. 1999, Murawski et al. 2001, Wiegand et al. 2007). Similar to fecundity, there are many factors affecting egg quality, which is defined as hatching success and survival probability. Some of these factors are the diet of the female, the size or weight of the oocyte, and the amount of nutrients, lipids and proteins deposited into the oocyte (Brooks et al. 1997). Mean protein and lipid content as a fraction of egg dry weight throughout a number of fish species were approximately 66.3% and 19.3%, respectively (Kamler 1992). These constituents make up the major energy source within the egg during embryonic development (Finn et al. 1995). Castro et al. (2009) accordingly showed for Peruvian anchoveta *Engraulis ringens* (Jenyns, 1842) that an increase in egg lipid and protein content enhance hatching success. Protein and lipid contents were also found to be positively related to enhanced nutritional condition in fish larvae (Guisande et al. 1998). Apart from their role as important source of metabolic energy for the developing embryo, lipids are an integral part in the formation of cell and organelle membranes, aid buoyancy control, and help waterproofing the integumentary system (Wiegand 1996). Knowledge on egg composition is therefore essential, since it has direct consequences on early life stage survival.

Assessing the composition and concentration of lipids in oocytes and larval tissue thus provides a good indication of egg quality and larval condition (Salze et al. 2005, Grote et al. 2011, Peters et al. 2015). Within lipids, fatty acids (FA) constitute an important sub group. The FA composition of oocytes is important for egg hatching success, proper development, and survival probabilities of early life history stages (e.g. Rainuzzo et al. 1997, Salze et al. 2005, Yanes-Roca et al. 2009, Tocher 2010, Migaud et al. 2013). Garrido (2007) gives a good introduction to the nomenclature of FA: "*FA are carboxylic acids with a long aliphatic chain, which is either saturated or unsaturated. FA have carboxyl and methyl ends, and their chains are lengthened by the addition of two carbon atoms at a time to the carboxyl end. The designation of FA is given according to the number of carbon atoms in*

the chain, the number of double bonds, and the inclusive number of carbon atoms from the terminal methyl to the carbon atom of the only or first double bond from the methyl end, also called the omega number. Therefore, 16:0 designates a FA with 16 carbon atoms and no double bonds, while 18:1 (n-9) designates a FA with 18 carbon atoms, 1 double bond and 9 carbon atoms from the methyl end of the molecule. Saturated fatty acids (SAFA) have no double bonds, monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds." The FA arachidonic acid (20:4 [n-6]; ARA), eicosapentaenoic acid (20:5 [n-3]; EPA), and docosahexaenoic acid (22:6 [n-3]; DHA) cannot be synthesized de novo in significant quantities by most marine heterotrophic organisms. Fishes, thus, need to acquire these FA through their prey, either consuming phytoplankton or feeding on zooplankton that in turn fed on phytoplankton (Ackman & Jangaard 1964, Lee et al. 1971, Sargent et al. 1995). For this reason, these FA are regarded as essential fatty acids (EFA). Not surprisingly, seasonal variations in plankton rapidly alter the body tissue and oocyte EFA composition of batch spawning fish (Shirai et al. 2002, Fuiman & Faulk 2013). Investigation of oocyte FA profiles provides a useful tool in approximating the survival probabilities of early life stages and to determine the reproductive potential of individual females as well as entire fish stocks especially in rapidly changing environments (Wiegand et al. 2007).

1.5. Climate Change

Clupeid fishes are likely to be highly sensitive to climate change since environmental fluctuations have direct effects on their metabolism. Changes of main prey production like phytoplankton and zooplankton that is controlled by climate variability, ultimately alter the carrying capacity for fish (Hays et al. 2005, Araújo et al. 2018, Henson et al. 2018). Increasing water temperatures will further alter FA profiles of marine phytoplankton (Hixson & Arts 2016), and thereby the ones of somatic tissues and reproductive products in clupeoid fishes (Shirai et al. 2002, Castro et al. 2010, Fuiman & Faulk 2013).

Due to a globally changing climate, the inversion of the salinity gradient in several estuaries throughout the dry tropics is either underway or can be expected in the near future (Wolanski 1986, Pagès & Citeau 1990, Ridd & Stieglitz 2002). Osmoregulation processes are energetically expensive and may interfere with a species' reproductive traits at elevated salinities (De Vlaming 1971, Sangiao-Alvarellos et al. 2003). In addition, phytoplankton community composition is also subject to changes under hypersaline conditions (Nche-Fambo et al. 2015).

Examination of sediment cores collected in West Africa suggested that coastal upwelling continues to intensify as global warming increases (Mcgregor et al. 2007). During the last years, equatorward winds increased in all upwelling ecosystems and a significant relationship between equatorward wind and

phytoplankton biomass could be observed (Demarcq 2009). Apart from this, variations in upwelling intensity were shown to alter diatom abundances (Bode et al. 2009). Cury and Roy (1989) observed a dome-shaped relationship between upwelling intensity and recruitment success in clupeid fishes. Hence, species-specific larval fish habitats are shaped by upwelling intensities (Tiedemann et al. 2017). Changing upwelling intensity regimes can have major impacts on the recruitment and feeding success as well as on the distribution of clupeid larvae.

In conclusion, alterations in water temperature, salinity in estuaries and upwelling intensity at the coast against the backdrop of a globally changing climate are suspected to have direct effects on the physiology and abundance of clupeid fishes and their planktonic food items (Egan et al. 2018).

1.6. The Bonga Shad *Ethmalosa fimbriata* (Bowdich, 1825)

Ethmalosa fimbriata (Bowdich, 1825) was chosen as a model organism for this thesis since various studies illustrated high phenotypic plasticity and adaptability in the species' reproductive traits in response to various environmental stressors (Scheffers et al. 1972, Albaret & Gerlotto 1976, Charles-Dominique & Albaret 2003, Guyonnet et al. 2003, Panfili et al. 2004, Panfili et al. 2006). The tropical, schooling species is distributed in West African Atlantic shelf waters from Mauritania to Angola, between 24°N and 12°S latitude (Charles-Dominique & Albaret 2003). *E. fimbriata* is of high economic importance for the entire West African region. It is one of the commercially most important fishes targeted by artisanal fisheries in Mauritania, Senegal, the Gambia, Ivory Coast, Cameroon, and Nigeria (Albaret & Charles-Dominique 1982, Moses et al. 2002, Charles-Dominique & Albaret 2003). Even though the species' annual total catch is fluctuating, in northwest Africa it exhibits a steadily increasing trend from around 23.000 tonnes in 1990 to 83.000 tonnes in 2014. Apart from a decline in total catches of *E. fimbriata* during the 2000s, catches in Senegal remained relatively stable with 23.000 tonnes in 1990 and 24.000 tonnes in 2014 (FAO 2015).

1.6.1. Morphology

E. fimbriata is a member of the family Clupeidae and the subfamily Alosinae. The morphometric characteristics of Alosinae are a terminal mouth and jaw teeth, which are small or absent. Strong scutes along the abdomen are additionally discriminatory for the subfamily (Nelson 2006). *E. fimbriata* is distinguished from other Alosinae species by its upper jaw with its distinct median notch into which the tip of the lower jaw fits (Charles-Dominique 1982).

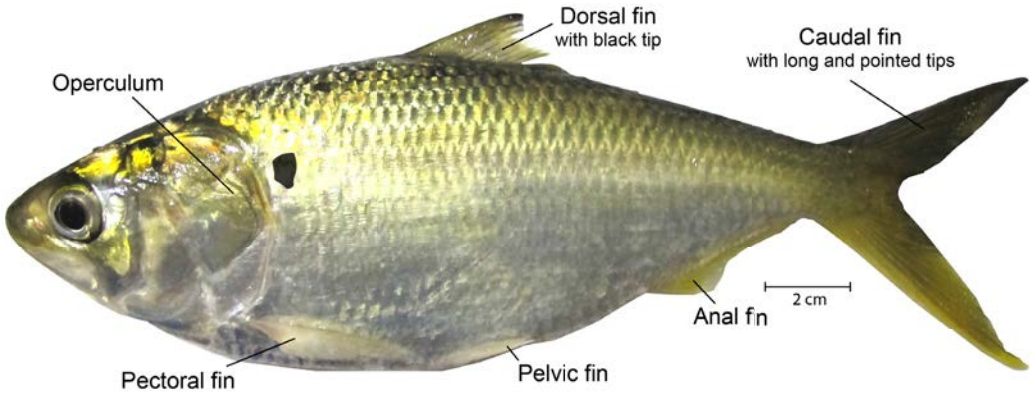

Figure 1.1 *Ethmalosa fimbriata* physiognomy at Djifer (Saloum River's mouth, Senegal)

The mouth is sharply bent upward and V-shaped. The lower gill rakers are long, fine, and numerous and often three times as long as the gill filaments, the upper gill rakers bent. The caudal fin is deep chrome with long and pointed tips, and the dorsal fin has a golden tint and a black tip (Figure 1.1). There is also an apparent dark spot behind the operculum, which is in some individuals followed by additional smaller spots (Abowei 2009). The species' maximum total length was reported to be 30.4 cm in Cape Coast (Ghana) (Blay Jr. & Eyeson 1982a) and 31.0 cm in Lagos lagoon (Nigeria) (Facade & Olaniyan 1972).

1.6.2. Reproductive biology

E. fimbriata has a salinity tolerance ranging from 0 up to 97 and is therefore regarded as euryhaline (Panfili et al. 2006). In different areas along the West African coast, *E. fimbriata* spawns either in the sea (Bainbridge 1961) or in estuaries and lagoons (Facade & Olaniyan 1972). When compared to the genetically identical Gambia population, both fecundity and oocyte size are higher in Sine Saloum individuals, indicating that a rather moderate salinity (< 60) is more favorable for *E. fimbriata* to reproduce (Panfili et al. 2004). Still, reproduction could be observed in waters with salinities up to 66 (Charles-Dominique & Albaret 2003). Past studies reported spawning in Senegalese coastal waters (St. Louis and Senegal River) and the Gambia River within a salinity range between 3.5 and 38 (Scheffers et al. 1972, Scheffers & Conand 1976). Further, Albaret and Gerlotto (1976) stated that the species spawns mainly in waters with salinities ranging between 18 to 26. However, highest larvae abundances in the Senegal River were observed at salinities ranging between 5 – 10 (Scheffers et al. 1972). Water temperature is the second physical factor severely affecting reproductive behaviour. For *E. fimbriata* Scheffers and Conand (1976) reported that temperatures of 22 – 23°C limited spawning in the Gambia River, and Albaret and Gerlotto (1976) reported a maximum spawning temperature of 30.2°C in Ivory Coast waters.

In the Sine Saloum estuary, a peak in gonado-somatic index was observed from January to September, hinting towards an intensified spawning activity in this period (Panfili et al. 2004). Still, a protracted spawning season in this population was proposed, lasting all year round (Panfili et al. 2006). Charles-Dominique (1982) recorded a spawning season from February to June in *E. fimbriata* specimens sampled at Joal (Senegal). Scheffers et al. (1972) observed all year round spawning with a minimum from December to February in St. Louis, Senegal. Diverging spawning seasons are described between Senegal (March to August), Sierra Leone (July to January), Ghana (October to March), and Nigeria (November to May) (Facade & Olaniyan 1972, Albaret & Gerlotto 1976, Blay Jr. & Eyeson 1982a). The species' reproductive period, thus, becomes progressively later towards the South (Froese & Pauly 2017).

Mean values for fork length at first maturity (L_{50}) determined in females in the Casamance River, the Gambia River, and the Sine Saloum estuary were 14.0 - 15.0, 18 - 19.1, and 15.3 cm, respectively (Scheffers et al. 1972, Scheffers & Conand 1976, Albaret 1987, Panfili et al. 2004). Scheffers et al. (1972) reported values for length at first maturity for the Senegalese coast (St. Louis; 17 cm). Still, these values are much higher than those observed in Ivory Coast lagoons, where they ranged between 8.4 and 14.5 cm (Guyonnet et al. 2003). Diverging values for L_{50} in *E. fimbriata* were linked to environmental pressures such as variances in water salinity (Panfili et al. 2004, Panfili et al. 2006). Different fish species also display a reduction in length and age at first maturity as an adaptive response to anthropogenic habitat disruptions (Smith 1994; Rochet 2000). Studies suggest that *E. fimbriata* responds to water pollution (Albaret & Charles-Dominique 1982, Guyonnet et al. 2003) as well as to overfishing (Laë 1997) with a reduction in this particular life history trait.

E. fimbriata is an iteroparous fish with asynchronous oocyte recruitment exhibiting pelagic indeterminate batch spawning (Albaret & Gerlotto 1976). The measurement of the number of eggs produced in a single spawning batch is, therefore, the only useful method to determine fecundity (Hunter et al. 1985). The absolute batch fecundity (*ABF*) of female *E. fimbriata* was calculated by Blay Jr. & Eyeson (1982a) for females sampled in Cape Coast waters, with 16,000 to 51,750 hydrated oocytes. This is rather low when compared to Lagos lagoon individuals, with 24,000 to 180,000 oocytes (Facade & Olaniyan 1972). The mean number of oocytes per female was 57,800 at Aiyetoro Coast, Nigeria. *ABF* ranged here extensively between 2,097 and 174,200 oocytes (Kusemiju & Onadeko 1990). Comparably low fecundities observed in Cape Coast females were explained with relatively poor feeding conditions (Blay Jr. & Eyeson 1982a, b). All mentioned studies on *ABF* confirmed the general trend, that fecundity is positively correlated with fish length and weight (Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982a, Hunter et al. 1985, Abowei 2009).

The relative batch fecundity (*RBF*) provides a mean to compare individuals of different populations through the standardization by female's body weight. Even though Blay Jr. & Eyeson (1982a) don't state, whether they assessed the females' gutted or ovary free body weight, recalculated *RBF* values roughly ranged between 182 to 213 oocytes g^{-1} spawner. This is quite similar to *RBF* values observed in the Gambia and Sine-Saloum estuary, where the mean values are 110 and 150 oocytes g^{-1} spawner, respectively (Panfili et al. 2004) and also in line with those reported for Ivory Coast females, ranging from 150 to 300 oocytes g^{-1} spawner (Albaret & Gerlotto 1976). Still, *E. fimbriata* seems to exhibit significantly higher batch fecundity when spawning in hypersaline conditions compared to marine or fresh waters (Scheffers et al. 1972, Albaret & Gerlotto 1976, Panfili et al. 2004).

Not only duration of spawning and fecundity, but also the total number of spawned batches and spawning frequency have to be taken into account for the determination of a fish stock's reproductive potential (Murua & Saborido-Rey 2003). Peak appearances of mature (stage V) females in Senegalese waters of 33% in February 1971, and of 40% in May 1972 were observed (Scheffers et al. 1972). A peak spawning fraction for the Gambia of 49% was reported in March 1974 (Scheffers & Conand 1976). Since the spawning frequency can be deduced from the inverse of the female spawning fraction (Jakobsen et al. 2009), it can be assumed that females in these regions are able to spawn every 2 – 3 days during the reproductive season.

After pelagic fertilization, the eggs of *E. fimbriata* spend 3 – 9 hours in the water column until hatching (Albaret & Gerlotto 1976). Incubation times in eggs of clupeid fishes, however, vary significantly with water temperature (Jatteau et al. 2017) and salinity (Holliday 1969). It was previously proposed that the eggs of *E. fimbriata* are spawned at sea and larvae subsequently drift into lagoons and estuaries by tidal action (Blay Jr. & Eyeson 1982a, b). No matter the place of spawning, estuaries appear to be important nursery areas for the species (Charles-Dominique & Albaret 2003, Ama-Abasi et al. 2005). In Nigeria, juveniles of *E. fimbriata* could be found in the Cross River estuary from November of each year to May (or even June). The authors further observed an emigration of juveniles out of the respective estuary system when the salinity exceeded 9. The authors suggested that the species' migration behaviour is correlated to phytoplankton abundances in the river, which decreased at higher salinity levels (Ama-Abasi et al. 2005).

1.6.3. Feeding ecology

During ontogeny, the species apparently switches between main prey items (Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982b, Gning et al. 2007). At first appearance in the Sine Saloum estuary, *E. fimbriata* larvae are zooplankton feeder, but as juveniles of 51 to 100 mm display a feeding preference for benthic invertebrates originating from the periphytic community in the vicinity of mangroves (Gning et al. 2007). Large quantities of sand, organic detritus,

and the presence of the diatom *Navicula* in the stomachs of juvenile fish also indicate that the species browses through benthic deposits (Bainbridge 1963, Facade & Olaniyan 1972). Adults are passive filter feeders (Lazzaro et al. 1987) and in estuarine environments mainly prey on diatoms of the genera *Actinocyclus*, *Coscinodiscus*, and *Stephanodiscus* (Bainbridge 1963, Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982b). Adults, however, may also occasionally feed on zooplankton like protozoan, crustacean, and molluscan larvae (Blay Jr. & Eyeson 1982b).

2. THESIS OBJECTIVES AND OUTLINE

One of the main goals of the project 'Ecosystem approach to the management of fisheries and the marine environment in West African waters' was to enhance the understanding of mechanisms defining the productivity of small pelagic fish (e.g. *Sardinella aurita*, *Sardinella maderensis*, and *Ethmalosa fimbriata*) populations in West African coastal waters. As described beforehand, one essential aspect in defining the productivity of clupeid fishes in space and time is in-depth knowledge on specialized reproductive tactics. Thus, in three chapters, the reproductive investment, the reproductive potential, and the stock structure of *E. fimbriata* will be investigated with special regard to environmental settings at the Senegalese South Coast and inside the Sine Saloum estuary. This thesis will contribute to the general understanding of reproduction processes and tactics of coastal clupeid fish species exposed to environmental alterations under climate change conditions.

Table 2.1 Contributions of PhD candidate to tasks within each research-based chapter. For each chapter, each listed contributing author was involved in the development of the final manuscript following the first draft.

Task	Contribution of PhD candidate [%]		
	Chapter I	Chapter II	Chapter III
Concept and design	90	100	90
Acquisition of data	100	100	100
Data analysis and interpretation	90	100	90
Preparation of figures and tables	100	100	100
Drafting of manuscript	100	100	100

Chapter I

Ethmalosa fimbriata (Bowdich 1825), a clupeid fish that exhibits elevated batch fecundity in hypersaline waters

Julian Döring, Maik Tiedemann, Moritz Stäbler, Hans Sloterdijk, and Werner Ekau

In the first chapter of my thesis I will investigate how a hypersaline environment influences the spawning biology of *E. fimbriata* females. Temperature and salinity may directly alter individual metabolic rates and osmoregulation efforts in clupeid fishes, and thus the energy that can be budgeted towards spawning (Hempel 1971, McBride et al. 2015, Döring et al. 2018a). I will therefore attempt to answer the following research question: **How is the species' reproductive biology adapted to cope with the extreme salinities prevalent in the Sine Saloum estuary?** More specific, it was attempted to uncover possible energetic trade-offs accompanied by inhabiting and spawning in hypersaline environments. Consequently, variations in condition index (as a proxy for somatic energy storage), gonado-somatic index (as a proxy for gonadal energy storage), as well as in batch fecundity of female *E. fimbriata* were investigated along a spatial and temporal temperature and salinity gradient at the Senegalese South Coast and within the hypersaline Saloum River.

Author Contributions: J.D. and W.E. developed the original research idea. J.D. designed the field sampling campaign. J.D. and H.S. carried out sample collection. J.D. performed the fecundity analyses. J.D., M.T., and M.S. analysed the data; J.D. wrote the paper with contributions from M.T. and W.E..

Chapter II

Using oocyte essential fatty acid composition to assess spawner reproductive potential under hypersaline conditions

Julian Döring and Werner Ekau

In the second chapter I will investigate oocyte fatty acid profiles to assess the reproductive potential of individual *E. fimbriata* females. Building on the results of Chapter I, I will address the research question: **Are the numerous oocytes to be spawned in the Saloum River potentially able to survive under increased salinity conditions?** The quality of reproductive products in terms of their fatty acid composition is important during the ontogeny of marine fishes (Watanabe 1993, Logue et al. 2000, Tocher 2010). Deficiencies in essential fatty acids may ultimately lead to impaired hatching success and lowered larval survival rates especially under hypersaline conditions (Pickova & Dutta 1997, St. John et al. 2001, Lane & Kohler 2006, Pickova et al. 2007). On top of this, alterations in environmental settings associated with a globally changing climate are suspected to have direct effects on the EFA composition of phytoplankton, *E. fimbriata*'s main food item (Bainbridge 1961, Blay Jr. & Eyeson 1982b, Hixson & Arts 2016). I will consequently investigate if oocyte quality in terms of fatty acid composition is related to the individual's reproductive investment (batch fecundity, gonado-somatic index, gonad weight, oocyte dry weight, oocyte volume) and influenced by ambient temperature and salinity conditions.

Author Contributions: J.D. developed the original research idea and designed the field sampling campaign. J.D. carried out the sample collection. J.D. performed the fecundity analyses. J.D. performed the fatty acid analyses. J.D. analysed the data. J.D. and W.E. wrote the paper.

Chapter III

Spawning energetics and otolith microchemistry provide insights into the stock structure of *Ethmalosa fimbriata*

Julian Döring, Carola Wagner, Maik Tiedemann, Patrice Brehmer, and Werner Ekau

Information on stock structure is crucial to unravel reproductive patterns and for enhancing productivity appraisal in marine fish populations. Exploited small pelagic fish stocks are often composed of different spawning components, and each component's contribution to the fished stock varies with its productivity (Begg et al. 1999). Less productive components are more vulnerable to exploitation than more productive ones (Jennings et al. 1998) and in order to improve fisheries management advice the relative contribution of a spawning component needs to be accurately determined. The question remains: **Are there different stock spawning components of *E. fimbriata* in southern Senegalese waters, and if yes, how does their productivity compare?** In Chapter III, I will therefore assess the stock structure of *E. fimbriata* in southern Senegalese coastal waters on the basis of spawning energetics/productivity in individual females and their otolith elemental fingerprint. For this purpose, the gross energy contents of spawning batches (fecundity, oocyte dry weight, oocyte lipid and protein content) and the microchemistry of sagittal otoliths in *E. fimbriata* will be compared between sampling sites at the Senegalese coast and inside the Sine Saloum estuary.

Author Contributions: J.D. and W.E. developed the original research idea. J.D. designed the field sampling campaign. J.D. carried out the oocyte and otolith sampling. J.D. performed the otolith elemental analysis at the Department of Geosciences, University of Bremen. J.D. analytically assessed the oocytes' protein and lipid content. J.D. and C.W. analysed the data. J.D. and M.T. wrote the paper with contributions from C.W., P.B., and W.E..

3. MATERIALS & METHODS

3.1. Study Area

For migratory clupeid fish species Senegalese coastal waters pose as a challenging spawning habitat due to their high variability in current patterns, temperatures, salinities, and primary productivity (Ndoye et al. 2017, Sloterdijk et al. 2017, Tiedemann & Brehmer 2017, Diankha et al. 2018). The Senegalese South Coast (SSC; 14°36'N to 13°36'N) is located in the southern part of the Canary Current Eastern Boundary Ecosystem, one of the most productive ecosystems in the world. The SSC harbours a seasonal upwelling cell, which governs ambient temperature fluctuations in the region. Because of the shelf's topography, northerly trade winds induce a strong tongue shaped upwelling core in winter/spring (Figure 3.1, Capet et al. 2017, Ndoye et al. 2017).

While in coastal regions with a narrow shelf the upwelling core is observed at the shelf break, the upwelling core at the SSC occurs on the shelf, where water depths are shallower than 100 m (Arístegui et al. 2009, Ndoye et al. 2014). In concert with sunlight, the upwelled cold and nutrient rich bottom waters facilitate the growth of phytoplankton which constitutes the basis of marine food webs (Auger et al. 2016).

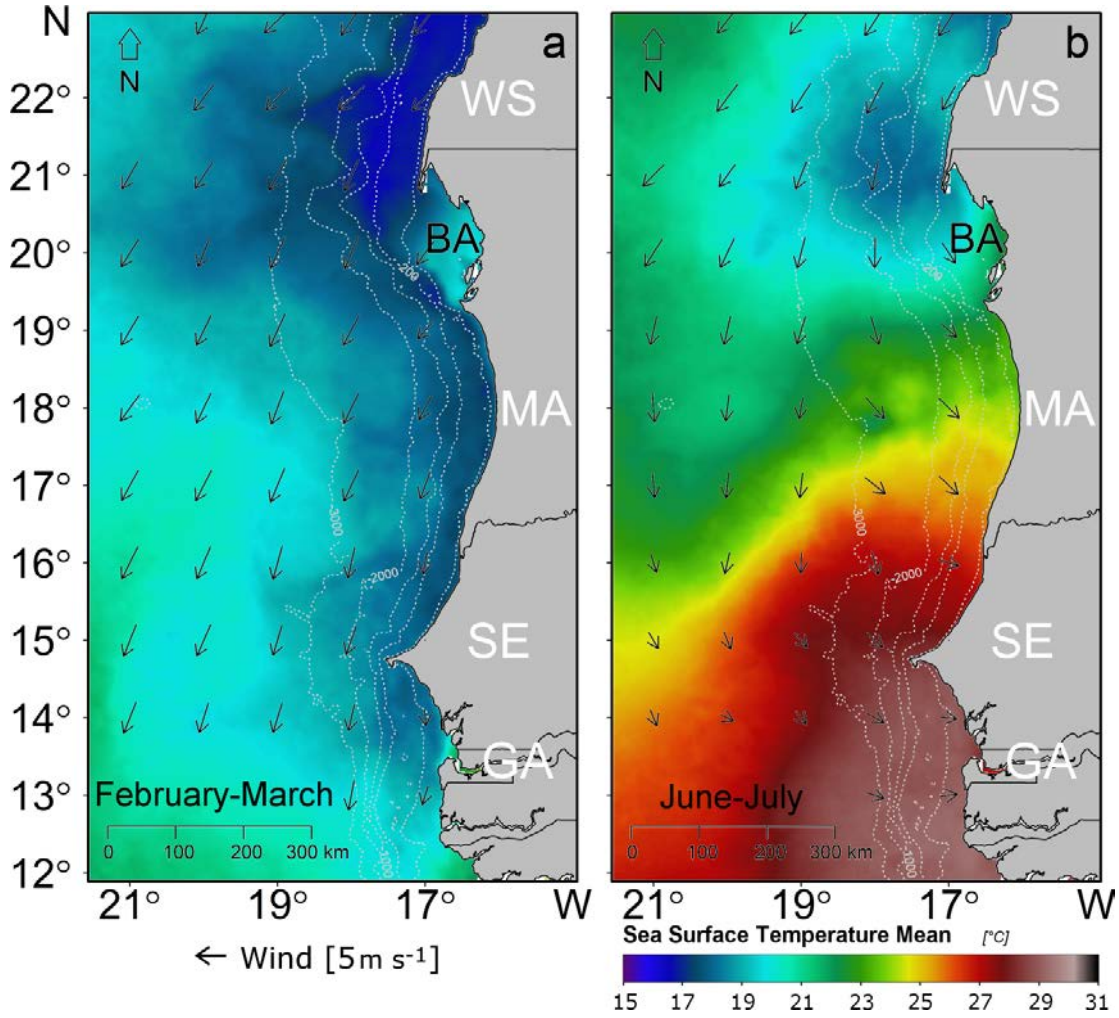

Figure 3.1 Sea surface temperature and surface wind regime derived from remote sensing and sampling stations during the cold season (a) and warm season (b) in 2014 along the southern part of the Canary Current Eastern Boundary Ecosystem. MO: Morocco; BA: Banc d'Arguin; MA: Mauritania; SE: Senegal; GA: Gambia (modified after Tiedemann et al. 2017)

The inverse (i.e. salinity increases upstream in all seasons) Sine Saloum estuary is located at the southern tip of the SSC (between 13°55' and 14°10'N and 16°03' and 16°50'W, Figure 3.2). The estuary is comprised of three main branches, the Saloum, the Diomboss, and the Bandiala. An extended dry season, with cool temperatures from November to March and warm temperatures from April to June, is characteristic for this region. July to October is considered the wet and warm season (Simier et al. 2004). Minimum water temperatures range between 21 and 22°C and are usually observed from December through February. Maximum reported values for water temperature are usually about 32°C in June and October (Saos & Pagés 1985, Diouf 1996, Panfili et al. 2004, Simier et al. 2004). The Saloum branch is 7 - 15 m deep; with a maximum water depth of 25 m (Saos & Pagés 1985). It is strongly influenced by 0.5 - 1.6 m semidiurnal tides that arrive at the upper reaches with a delay of around seven hours (Simier et al. 2004). The main

causes of the estuary's hypersalinity were given by Pagès & Citeau (1990) and include a small catchment, which is located in an area of low precipitation; a low land elevation relative to sea level (hypsometry), causing a very low run-off coefficient, and leading to little fresh-water discharge; a negligible slope of the river at the estuary's mouth, which allows for sea-water intrusion; a very shallow mean depth, which amplifies the precipitation deficit; and high evaporation and low rainfall. In the estuary's upper reaches, water salinity thus regularly surpasses 130 by the end of the dry season in June/July (Simier et al. 2004). Mangrove forests cover almost the entire southern part of the system and successively diminish going North. Mangrove cover is luxuriant in the Bandiala, patchy in the Diomboss and in the lower part of the Saloum. Because of high water salinities the mangrove cover completely disappears in the upper reaches of the Saloum River (Figure 3.2 a, b; Simier et al. 2004, Trape et al. 2009, Sloterdijk et al. 2017).

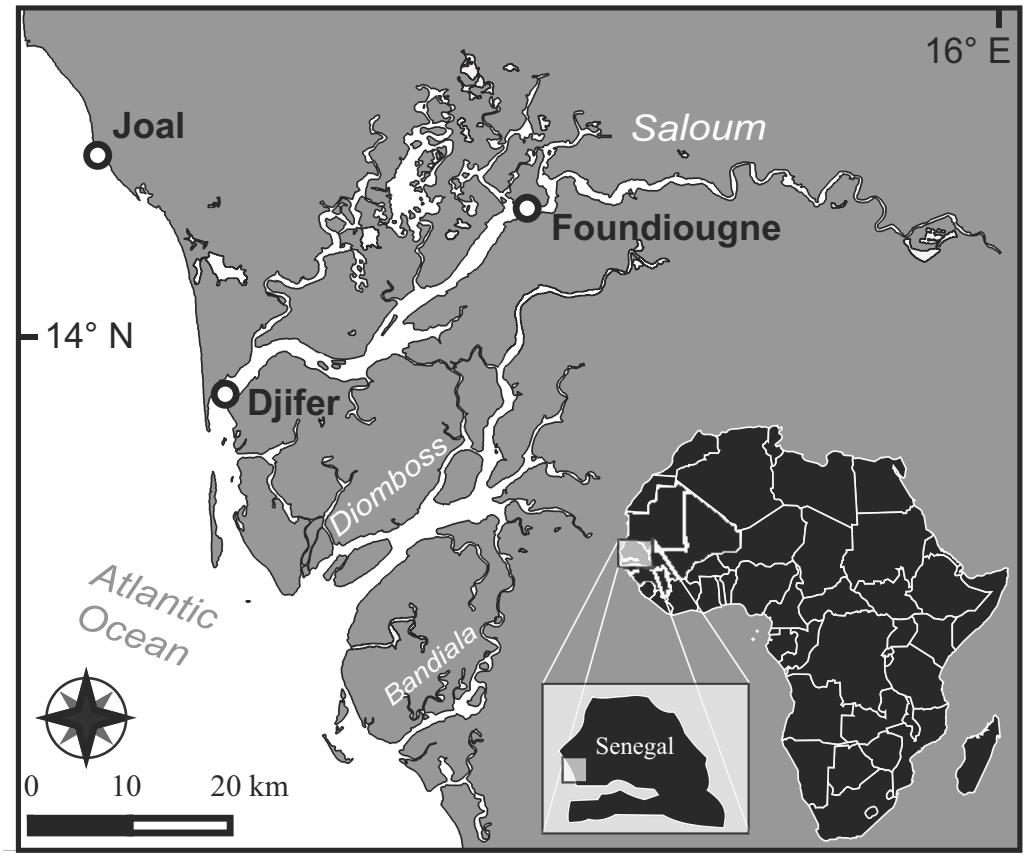

Figure 3.2 Map of the Sine Saloum estuary at the Senegalese South Coast with sampling sites Joal, Djifer, and Foundiougne

Given the vast range in environmental settings encountered in the SSC, the region poses as a suitable study area to investigate how clupeid fishes adapt their reproductive tactic in order to cope with spatial and seasonal changes in their spawning habitats. Due to a globally changing climate the inversion of estuaries in other parts of the dry tropics can be expected in the near future (Wolanski 1986, Pagès & Citeau 1990, Ridd & Stieglitz 2002).

Figure 3.3 The thick mangrove cover and African baobab trees at the mouth of the Sine Saloum (a), salt covered floodplains at the estuary's upper reaches during dry season (b), the estuary's mangrove forest at sunset (c)

3.2. Environmental Parameters

To get an overview on seasonal changes of environmental conditions, water temperatures, and salinity were measured. Because the upwelling area in front of Senegal (mean depth <20 m) and the Saloum River (mean depth <10 m) are well mixed water bodies, surface water temperatures and salinities were employed (Saos & Pagés 1985, Sloterdijk et al. 2017, Tiedemann & Brehmer 2017). Satellite-derived (Moderate-resolution Imaging Spectroradiometer – MODIS Aqua, level 2, 0.1 degrees) sea surface temperatures (https://oceancolor.gsfc.nasa.gov/) were assessed for the SSC and for the Saloum River's mouth once per sampling week. As no remote sensing data for inland waters were available, the Saloum River's surface water temperatures were recorded in situ once per sampling week with a digital thermometer (ama-digit ad 15th; precision 0.4%; accuracy 0.4%). For all stations, salinity was measured with a handheld refractometer (Aqua Medic; precision 0.7%, accuracy 0.2%)

using the Practical Salinity Scale (PSS-78). Monthly means in MODIS satellite-derived (Aquarius, level 3, 0.5 degrees) sea surface salinities (https://oceancolor.gsfc.nasa.gov/) were used to double-check the coastal ocean's salinity.

3.3. Sample Collection

3.3.1. Fish sampling

Monthly sampling was conducted at the SSC and inside the Saloum River from February to October 2014, during the peak of *Ethmalosa fimbriata*'s extended spawning season (Charles-Dominique 1982, Panfili, Durand, et al. 2004). Three environmentally contrasted study sites were chosen: Joal (SSC, 14°9.1' N; 16°51.7' W), Djifer (Saloum River's mouth, 13°57.8' N; 16°44.8' W), and Foundiougne (Saloum River's middle reaches, 14°8.1' N; 16°28.1' W) (Figure 3.2). Fish were caught with gill nets (32 - 36 mm mesh size) by local fishermen (Figure 3.4 c**)**, and immediately stored on crushed ice after landing. Staging of fish was conducted macroscopically after a key by Blay Jr. & Eyeson (1982). Discrimination between mature females with ovaries containing fully hydrated oocytes (stage V) and females that have recently spawned (spent, stage VI) were based on West (1990) and ter Hofstede et al. (2007). In order to calculate female spawning fractions the number of running ripe females was set in relation to all females in a subsample of 100 fish (Claramunt & Roa 2001).

To ascertain *E. fimbriata*'s batch fecundity, ca. 1000 fish per sampling site and month were examined in order to find spawning ready females. Running ripe females were not sampled, since they might have released part of the egg batch.

Figure 3.4 Dissecting an *Ethmalosa fimbriata* specimen in Foundiougne (Sine Saloum, Senegal) (a), the species' hydrated oocytes (b), a small fishing village at the Saloum River's bank, including fishing pirogues and shell mound (c)

3.3.2. Oocyte sampling

Oocytes were carefully extracted from one ovary lobe, rinsed with deionized water, and counted under a stereomicroscope (Figure 3.4 b). Around 70 oocytes per fish were transferred to a pre-weighed tin cap, and deep frozen in liquid nitrogen for oocyte fatty acid and lipid determination. Around 10 oocytes per female were transferred to a pre-weighed tin cap, and deep-frozen in liquid nitrogen for oocyte protein determination.

3.4. Reproductive Traits

3.4.1. Spawner length

Fish were measured by fork length to avoid falsifications due to fin damage. Accordingly, 100 fish with intact caudal fins were measured twice, by fork length as well as total length, to obtain a length coefficient for subsequent length data transformation. Conversions of fish fork length (L_F, nearest mm) to total length (L_T) were conducted using the equation $L_T = 1.0007 \times L_F + 3.9$ (Student's t-test, $p < 0.05$, $r^2 = 0.83$).

3.4.2. Somatic and gonadal energy storage

In order to assess the nutritional status of immature female fish (Stage I – IV), a condition index (K) (Equation 3.1) was calculated for each individual using wet weight (W_W, ±0.1 g), total length (L_T) (Lloret et al. 2014), and b of the length-weight relationship (3.62, CHAPTER I). The condition index (K_{hyd}) (Equation 3.2) and gonado-somatic index (I_G) (Equation 3.3) of hydrated female spawners (Stage V) was based on the ovary-free body weight (W_{OF}, ±0.1 g) and the ovary weight (W_{Ov}, ±0.0001 g) (Zydlewski et al. 2003). These indices were used as proxies for the somatic and gonadal energy storage of female spawners.

$$K = \frac{W_{\mathrm{W}}}{{L_{\mathrm{T}}}^{\mathrm{b}}} \times 1000 \tag{3.1}$$

$$K_{hyd} = \frac{W_{OF}}{{L_T}^{b}} \times 1000 \tag{3.2}$$

$$I_{\mathrm{G}} = \frac{W_{\mathrm{Ov}}}{W_{\mathrm{OF}}} \times 100 \tag{3.3}$$

3.4.3. Fecundity

E. fimbriata is an indeterminate batch spawner (Albaret & Gerlotto 1976). Therefore, measuring the number of hydrated oocytes produced in a single spawning batch is the only useful method to determine fecundity (Hunter et al. 1985). Absolute batch fecundity (*ABF*) was consequently assessed gravimetrically using the hydrated oocyte method for indeterminate spawners (Alheit 1993, Olney & McBride 2003, Haslob et al. 2011). *ABF* was divided by W_{OF} to calculate the relative batch fecundity (*RBF*) (Alheit 1988). Due to high variability in *E. fimbriata*'s batch fecundities, we re-analysed the 10% of the sampled ovaries containing the least amount of oocytes. We measured the diameters of seemingly hydrated oocytes within the questionable samples and compared these values with diameter values of hydrated oocytes stored in ovaries with high absolute batch fecundities, which were sampled in the same months, using ANOVA (analysis of variance) techniques. Ovaries containing oocytes with significantly lower diameters were rejected. Only hydrated oocytes with a diameter greater than 0.80 mm were taken into account for fecundity analysis. Additionally, ovaries containing oocytes without significant differences in diameter were re-examined macroscopically.

3.4.4. Oocyte volume

Oocyte volume was determined since it influences many features of the offspring and has an important effect on early life stage growth and survival (Brooks et al. 1997, Leal et al. 2009). Ten oocytes per ovary fixed in 4% formalin were photographed under a stereomicroscope and measured along their minor (d_1) and major (d_2) diameters using the calibrated measurement tool in Image J 1.8.0 (http://rsb.info.nih.gov/ij/). Following Alderdice et al. (1979), subsequent equation was employed to calculate oocyte volumes (*OV*, mm^3) (Equation 3.4):

$$OV = \frac{4}{3}\pi\left(\frac{d_1}{2}\right)^2\left(\frac{d_2}{2}\right) \quad (3.4)$$

3.4.5. Oocyte fatty acid composition

The fatty acid (FA) composition of oocytes is especially important for egg hatching success, proper development, and thus survival probabilities of early life history stages of marine fishes (Brooks et al. 1997). Knowledge on oocyte FA composition therefore allows for determination of a female's reproductive potential (Wiegand et al. 2007). Lipid extraction was performed using a 2:1 solvent mix of dichloromethane/methanol. The deuterated FA 12:0 (Larodan, 20 ng μl^{-1}) was added as an internal standard and a deuterated 22:0 FA standard (Larodan, 20 ng μl^{-1}) as an esterification efficiency control. Samples were homogenized using ultrasound. Esterification was performed for 8 h at 80°C in 250 µl hexane and 1 ml 3% sulphuric acid/methanol solution. All solvents used were gas chromatography (GC) grade. The fatty acid methyl esters (FAME) were analysed in 1 ml aliquots injected in an Agilent Technologies 7890B GC system equipped with a polar column (Macherey-Nagel, OPTIMA 225, 30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (base temperature 260°C) with helium as a carrier gas at a constant flow of 1 ml min^{-1}. The column oven was initially set to 40°C and then heated at a 100°C min^{-1} increase to 112°C for 2 min, at 14°C min^{-1} to 125°C, at 5°C min^{-1} to 160°C, at 20°C min^{-1} to 180°C, at 3°C min^{-1} to 200°C, at 4°C min^{-1} to 240°C for 15 min, and at 20°C min^{-1} to 260°C, which was held for 5 min. The system was calibrated with a 20 component marine oil FAME mix (Restek), and chromatograms were analysed using MassHunter Work Station - Quantitative Analysis (Agilent, Version B.07.00) GC software. Three random samples were processed via GC mass spectrometry (Macherey Nagel, OPTIMA FFAplus, 30 m × 0.25 mm × 0.25 µm, with identical oven parameters as above) to verify FA profiles. To ensure comparability with past studies, results for FA are given as a percentage of the combined weights of all detected FA (Castro et al. 2010).

3.4.6. Oocyte lipid and protein content

Lipid and protein make up the major energy source within the egg during embryo growth and their quantities inside the oocyte are closely linked to larval hatching success (Finn et al. 1995, Castro et al. 2009). Further, spatio-temporal changes in oocyte composition allow for deeper insights into spawning tactics in fish populations (Castro et al. 2009, Döring et al. 2018a). The total lipid content was assessed via summation of the weights of all detected fatty acids. For protein analyses, counted oocytes in tin caps were dried at 40°C for >24 h and weighed again for dry weight determination. Total organic carbon and nitrogen (N) content was measured using a EuroVector EuroEA3000 Elemental Analyzer. From the total amount of N in the sample, the protein content was calculated according to Kjeldahl (Bradstreet 1954), using a nitrogen-protein conversion factor of 6.25.

3.4.7. Spawning batch energy content

The cost of reproduction is an important issue in bioenergetics modelling, but data on the amount of energy invested into spawning by a female fish are not available for most species (Riis-Vestergaard 2002). An individual female's ability to invest energy into reproduction is closely coupled with a stock's

reproductive potential and with its productivity (Pecquerie et al. 2009). The oocyte gross energy content (J) was calculated on the basis of measured protein and lipid content, which were multiplied by corresponding energy values from literature: The amount of proteins per given oocyte (P, mg) was multiplied by a factor of 23.66 J mg^{-1} and subsequently added to the total amount of lipids per oocyte (L, mg) multiplied by 39.57 J mg^{-1} (Henken et al. 1986). Dividing the oocyte energy content by oocyte dry weight allowed for calculation of the oocyte's calorific value (J mg^{-1}).

Further, the oocyte energy content of each individual *E. fimbriata* female was multiplied by its respective relative batch fecundity (*RBF*) in order to obtain a standardized estimate of the total amount of energy invested into a single spawning batch per unit body weight (*SBEC*, J g^{-1} W_{OF}) (Döring et al. 2018a) (Equation 3.5).

$$SBEC = \left[\left(\mathrm{P} \times 23.66\ \frac{\mathrm{J}}{\mathrm{mg}}\right) + \left(\mathrm{L} \times 39.57 \frac{\mathrm{J}}{\mathrm{mg}}\right)\right] \times RBF \qquad (3.5)$$

3.4.8. Otolith microchemistry

Discrimination between stock components on the basis of otolith elemental composition is a widely used methodology (e.g. Kerr & Campana 2013, Avigliano et al. 2017). The chemical composition of otoliths is a natural marker of habitat use due to the otolith's continuous growth throughout the fish's lifetime and its metabolic inertness (Campana & Neilson 1985). Sagittal otoliths were extracted, rinsed with ethanol (70%), and stored dry in Eppendorf caps. Dried sagittal otoliths were embedded in epoxy resin (Araldite 2020; Huntsman, USA) on glass slides. They were ground from the proximal side down to the nucleus using an MPS2 surface-grinding machine (GN, Nürnberg, Germany) and polished with a diamond-grinding wheel (grain size 15 µm). Concentrations of eight elements (Mg, Mn, Cu, Zn, Sr, Y, Ba, and Pb) were determined along transects of up to 2000 µm length along the rostrum's anterior edge on the otolith's proximal side (Figure 3.5). Analyses were carried out by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) using a NewWave UP193 solid-state laser coupled to a Thermo-Finnigan Element2 ICP-MS. The employed analytical procedure used a pulse rate of 10 Hz, an irradiance of ca. 1 GW cm^{-2}, a spot size of 75 µm, and an ablation speed of 5 µm s^{-1} (for details see Marohn et al. 2011).

For external calibration the glass reference material NIST610 was analysed after every second ablation path, using reference values of Jochum et al. (2011). Data quality was validated by analysing a pressed pellet of NIES22 otolith (Yoshinaga et al. 2000) as well as through regular analyses of BCR-2G and BHVO-2G standard glasses (Jochum et al. 2005). For standard measurement results see Appendix Table B. Precision was <2% and the accuracy was <6% for Zn. To account for the substitution of Calcium (Ca) by the divalent elements Ba, Sr, and Zn all element concentrations are given as element:Ca ratios (Campana 1999). Only mean otolith Ba:Ca, Sr:Ca, and Zn:Ca ratios were employed in order to discriminate between spawning sites. Differences in these element:Ca ratios are the ones most commonly examined in studies on shad migration behaviour and stock delineation (e.g. Limburg 1995, Secor & Rooker 2000, Magath et al. 2013, Avigliano et al. 2017, Rohtla et al. 2017).

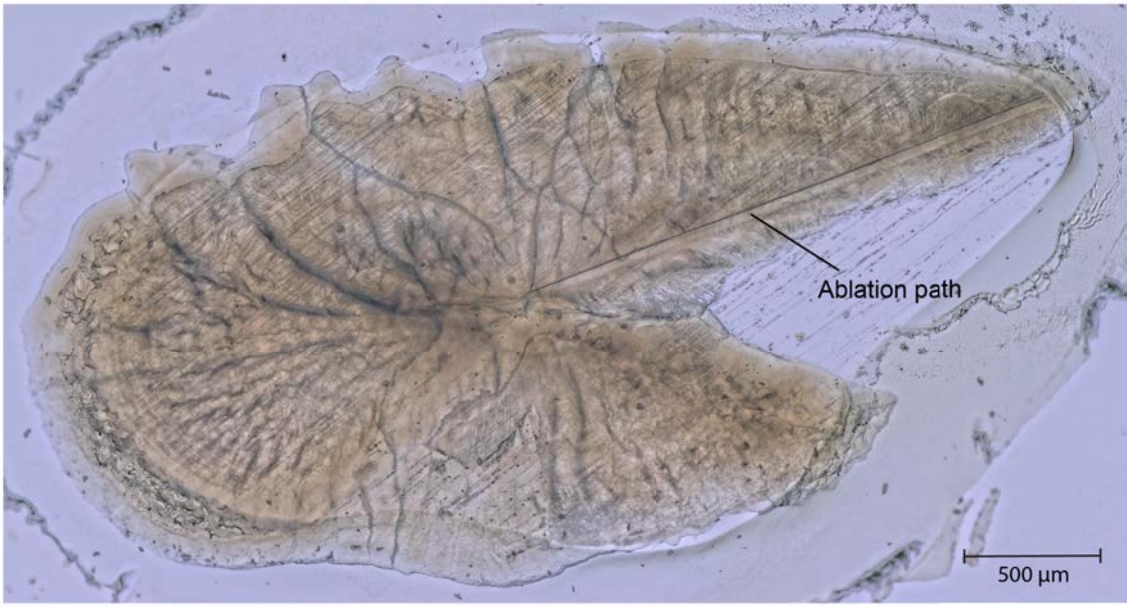

Figure 3.5 Ground otolith embedded in epoxy resin of a female *Ethmalosa fimbriata* specimen (23.1 cm L_T) sampled at Foundiougne (Sine Saloum, Senegal) in May 2014. The ablation path is clearly visible along the edge of the otolith's rostrum

3.5. Disclaimer

In fisheries biology, the mass of an object is most commonly referred to as its weight, even though these are in fact different concepts and quantities. In the strict sense of the word, mass is both a property of a physical body and a measure of its resistance to acceleration (Jammer 2000). Weight, on the other hand, refers to the local force exerted on an object by gravity (Morrison 1999). If an object on Earth is not moving, the values of mass and weight will be the same. If you change the object's location with respect to gravity, its mass will remain unchanged, but its weight will vary. In this thesis the term weight was favoured over mass, in an effort to stay consistent with the cited literature.

4. SYNOPTIC DISCUSSION

Clupeid fishes have developed specialized reproductive tactics in response to hydrographic fluctuations in their spawning habitats. Unprecedented environmental conditions induced by on-going climate change will challenge these behavioural and physiological adaptations. In the case of maladaptation coupled with no genetic evolution, shifts in clupeid distribution will be undermined and changes in stock sizes are foreseeable (Lambert et al. 2017). Taking into account spatiotemporal reproductive behaviour, reproductive value with regard to spawner age/length, reproductive potential, and stock structuring, this work aimed at assessing the reproductive tactics of a clupeid fish against the backdrop of globally changing climate. Obtained results will contribute to the understanding of recruitment variability in clupeid fish stocks reproducing in stressful environments throughout the dry tropics.

4.1. Phenotypic Plasticity

Organisms are expected to display diverse responses to climatic changes, including shifts in species distribution, changes in phenology, and physiological modifications (Hughes 2000). These responses are mainly based on mechanisms of phenotypic plasticity. The term phenotypic plasticity describes the ability of an organism with a given genotype to produce different phenotypes in response to environmental changes (West-Eberhard 1989, Pigliucci 1996). *Ethmalosa fimbriata* (Bowdich, 1825) expressed high phenotypic plasticity in all reproductive traits examined in past studies such as timing and duration of spawning, spawning intensity, length at first maturity, and batch fecundity (Scheffers et al. 1972, Albaret & Gerlotto 1976, Charles-Dominique & Albaret 2003, Guyonnet et al. 2003, Panfili et al. 2004, Panfili et al. 2006, CHAPTER I). The species further exhibited high variations in the oocyte traits investigated in the studies conducted within the scope of this thesis, in the like of dry weight, protein and lipid content, energy content, and fatty acid (FA) composition (CHAPTER I, CHAPTER II). The observed high variances in oocyte FA composition might further be due to adaptations in the species' feeding preferences or migratory behaviour (Crawford et al. 1986, Fréon et al. 1997, Fuiman & Faulk 2013).

4.2. Reproductive Behaviour

For a mature fish, a successful reproductive tactic involves spawning during environmental conditions, which warrant offspring survival (Hjort 1926, Roy et al. 1989, Bakun 1996). Since environmental conditions vary during the year, mature fish have adapted towards optimizing reproductive behaviour based on season and location (Cury & Roy 1989). In accordance with ocean processes, clupeid fishes have further developed specific spawning

behaviours. These processes may be the occurrence of coastal upwelling events, emergent mesoscale processes beneficial for dispersal, or the establishment of rich food supply through plankton blooms (Cushing 1969, Platt et al. 2003, Tiedemann & Brehmer 2017, Tiedemann et al. 2017, 2018). In clupeids inhabiting estuarine environments, migratory behaviour and spawning are triggered by cues such as water temperature, photoperiod, and riverine discharge to ensure early life stage survival in these highly variable habitats (Quinn & Adams 1996, Lambert et al. 2017).

Past studies on the reproductive behaviour of *E. fimbriata* reported peak reproduction in the Atlantic Ocean from February until June (Charles-Dominique 1982), and inside the Saloum estuary from January until September (Panfili et al. 2004) or even all year round (Panfili et al. 2006). These observations are in line with the occurrence of spawning ready (stage V) females during the sampling period (Figure 4.1). Locally varying spawning times, therefore, hint towards favorable hydrographic conditions for offspring survival at the Senegalese South Coast during the upwelling season (Ndoye et al. 2014). From February to July, retention rates over the southern Senegalese shelf are highest, and a similarly timed spawning tactic in round sardinella *Sardinella aurita* (Valenciennes, 1847) was proposed to result from a trade-off between beneficial retention patterns and food availability (Mbaye et al. 2015). At the Saloum River's mouth, reproduction in *E. fimbriata* could be observed until July. Here, hydrographic conditions were quite stable until mid-year and the species seemed to aim at spawning before salinity fluctuations and riverine discharge pose as stressors in the system's mouth (CHAPTER I).

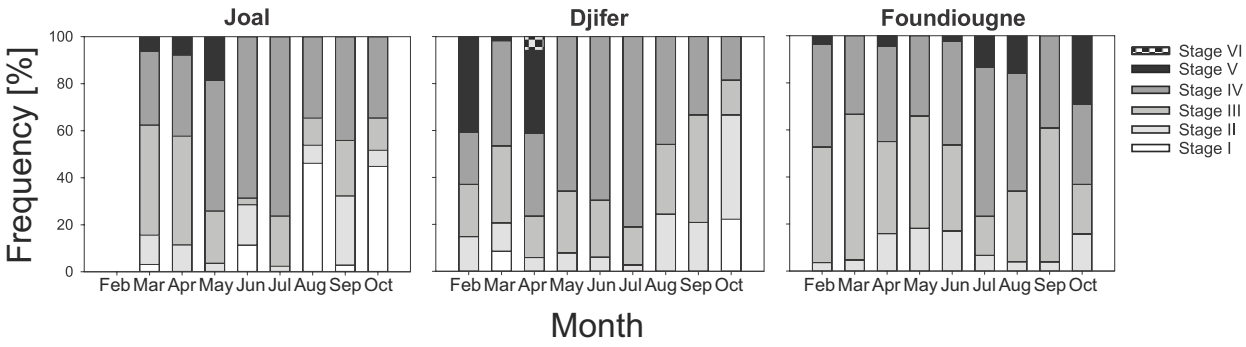

Figure 4.1 Progression of maturity stages in female *Ethmalosa fimbriata* throughout the sampling period in 2014 at the three sampling sites: Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches). Stage I: immature, Stage II: sexual resting, Stage III: late developing, Stage IV: ripe, Stage V: spawning, Stage VI: spent

Shads prefer upper and midriver regions for spawning (Bilkovic et al. 2002), and judging by *E. fimbriata*'s prolonged spawning season, conditions are favorable for spawning and early life stages survival in the Saloum River's middle reaches all year around. It is worth noting, that a prolonged spawning

period may further be a valuable adaptation to the system's less-predictable hydrography (Powell 1994). Temporal fecundity variations additionally hint towards intensified spawning activity in the Saloum River's middle reaches at the end of the wet season (CHAPTER I, CHAPTER II, CHAPTER III).

Fecundity in teleosts is known to vary temporally and spatially in relation to parental wealth (e.g. weight and condition), food availability (e.g. abundance and quality), environmental (e.g. temperature and salinity) and evolutionary stressors (e.g. stock density and fishing pressure) (Lambert 2008, McBride et al. 2015). All of these parameters were previously shown to affect fecundity in clupeid fish species such as Baltic sprat *Sprattus sprattus* (Linnaeus, 1758) (Alheit 1988, Müller et al. 1990, Alekseev & Alekseeva 2005, Haslob et al. 2011, 2013) and Pacific herring *Clupea pallasii pallasii* (Valenciennes, 1847) (Ware 1980, Tanasichuk & Ware 1987, Hay & Brett 1988). Batch fecundity in *E. fimbriata* is highly variable (Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982a, Kusemiju & Onadeko 1990; CHAPTER I), which seems to be a characteristic commonly shared in shads (Olney & McBride 2003, Hyle et al. 2014, Lambert et al. 2017). This feature might be a valuable adaptation to react to rapid fluctuations in the spawning environment at an individual level (Leggett & Carscadden 1978).

Fecundity variations in *E. fimbriata* could be linked to the highly variable hydrological conditions throughout southern Senegalese estuarine and coastal waters. The species exhibited elevated fecundity in the Saloum River, at water temperatures (26 - 30°C) and salinities (42 - 51), which by far exceed marine conditions (Figure 4.2 a). To our knowledge, this thesis incorporates the first field study to evidence elevated batch fecundity exhibited by a teleost fish in hypersalinity, when compared to marine conditions. Peak fecundity was observed at water temperature and salinity ranges that are predominant in the Saloum River's middle reaches during the end of the rainy season (Pagès & Citeau 1990). The species' spawning tactic may, therefore, aim towards spawning in a favourable environmental window (Motos 1996), ensuring the survival of well-adapted offspring through enhanced quality in food items and/or predator avoidance during the consecutive weeks (May 1974).

While in clupeid fishes somatic condition is generally positively correlated with fecundity (Milton et al. 1995, Haslob et al. 2013, Döring et al. 2018a), in *E. fimbriata* a negative relationship between these two attributes could be observed (Figure 4.2 a, b). Individual females invest substantial amounts of energy towards elevated metabolic rates, iono-osmoregulation, and spawning to reproduce in the prevalent hypersaline conditions (McCormick et al. 2012, CHAPTER I). The ability to spatially and temporally adjust fecundity may further be a valuable adaptation to variable spawning habitats and to alternations in food availability (Leggett & Carscadden 1978, Lowerre-Barbieri et al. 2015, McBride et al. 2015). Nevertheless, fecundity variations in

clupeoids often accompany trade-offs in oocyte quality (Jessop 1993, Leal et al. 2009, Döring et al. 2018a).

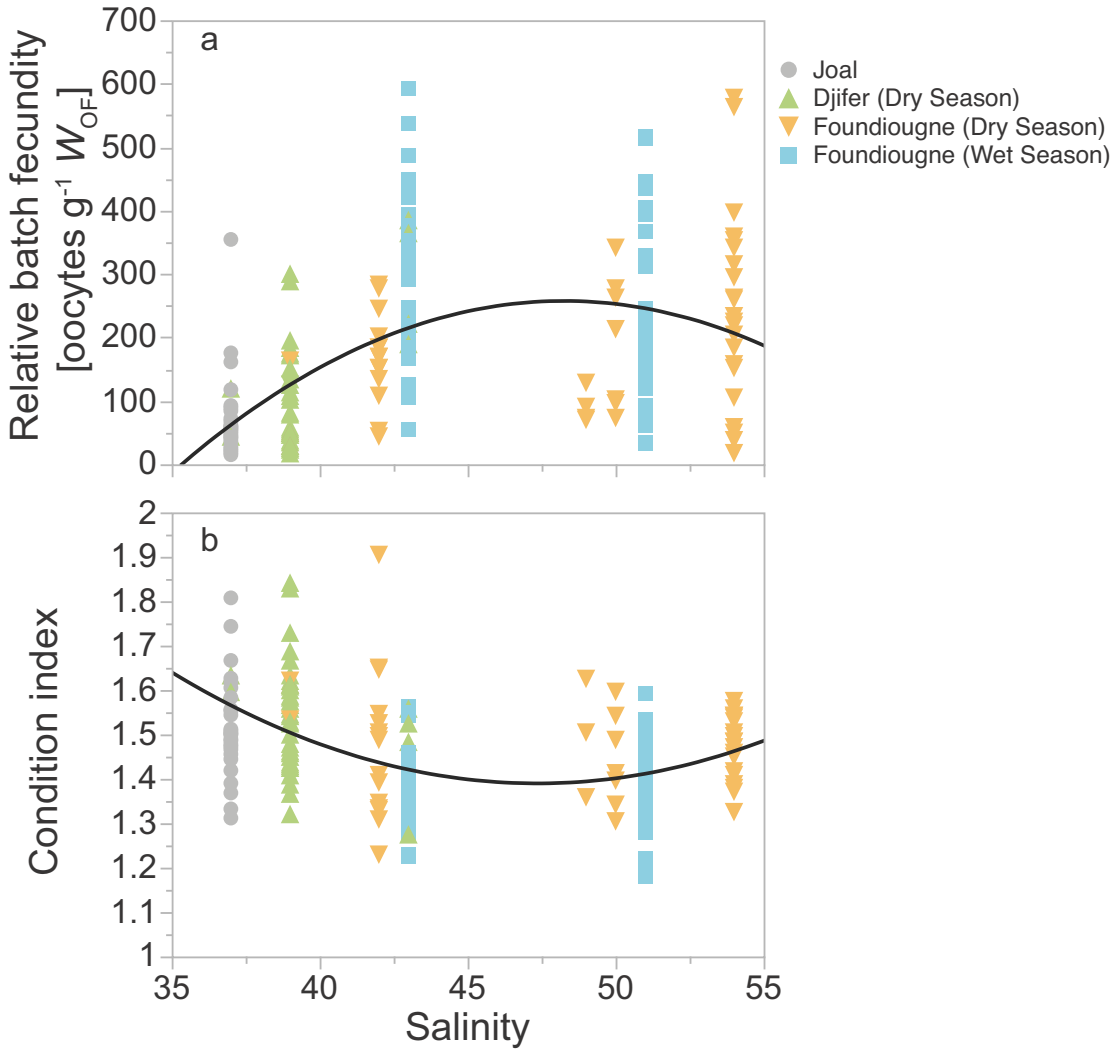

Figure 4.2 Relationships between (a) relative batch fecundity and (b) condition index with surface water salinity. Female *Ethmalosa fimbriata* were sampled at Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches) in February to October 2014. W_{OF}: Ovary free body weight

4.3. Reproductive Value and Spawner Age

In teleosts, oocyte size or volume is commonly used as a proxy for quality and trade-offs between fecundity and oocyte size are often observed (Milton et al. 1995, Castro et al. 2009, Closs et al. 2013). The volume of an oocyte may not be a suitable indicator for quality, however, since various factors (e.g. size of female's body cavity and egg buoyancy) may interfere with this trait (Chambers 1997, Shi et al. 2008). West (1990) suggested that in small fish with short life histories, narrow ranges in adult length and egg size may obscure the

relationship between female size and egg size. Oocyte volume was found to be a conservative trait in *E. fimbriata* across all female size classes and encountered environmental settings (CHAPTER II). Our results, however, support the assertion that the dry weight of an oocyte is negatively correlated with batch fecundity in clupeid fishes (Bradford and Stephenson 1992; Jessop 1993; CHAPTER II). Oocyte lipid and protein content were further positively linked to oocyte dry weight, and oocyte energy content was therefore negatively correlated with batch fecundity (CHAPTER II). *E. fimbriata* produced, thus, higher numbers of hydrated oocytes, albeit of lower energy content, in the Saloum River's middle reaches during the rainy season (August – October) (CHAPTER I, CHAPTER II, CHAPTER III).

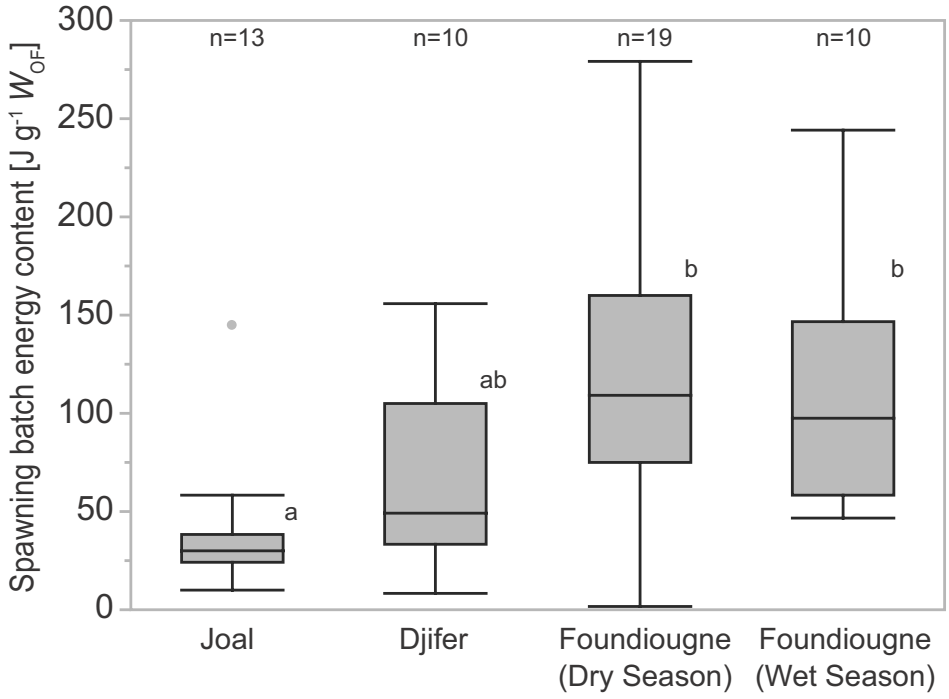

Figure 4.3 Spatial and seasonal differences in spawning batch energy content in *Ethmalosa fimbriata* females sampled at the Senegalese south coast (Joal), at the Saloum River's mouth (Djifer), and inside the Saloum River's middle reaches (Foundiougne) from March to October 2014. Whiskers show the 5th and 95th percentiles, solid lines indicate median values, and outliers are marked as dots. Differences between stations were significant if respective boxplots do not share the same letter (Tukey HSD). W_{OF}: Ovary free body weight

The female's ability to invest energy into spawning may change in the course of the spawning season even when environmental conditions remain unaltered. This is due to physiological shifts in energy allocations during the reproductive season (Castro et al. 2010). Even further, the surplus energy available to the female for spawning changes in respect to heterogeneous environmental conditions at the spawning sites because of metabolic constraints and fluctuations in food availability (McBride et al. 2015). Accessory

reproductive activities such as spawning migrations also compete with the allocation of surplus energy to gonad development (Leggett & Carscadden 1978, Leggett & Trump 1978, Glebe & Leggett 1981, Navodaru & Waldman 2003). With increasing female length the energy invested into growth decreases, allowing more energy reserves to be budgeted towards reproduction (Houde 1989, Sogard 1997, Marshall & Browman 2007, Gislason et al. 2010). The energy available for spawning may, thus, be highly variable over the length of the spawning season and throughout the female's lifetime. Energy allocation and physiological budgeting may, therefore, shape reproductive tactics in small pelagic fishes (Pecquerie et al. 2009, McBride et al. 2015). In our study, the energy invested into reproduction by individual females remained unchanged during the spawning period and throughout female size classes, signifying no effect of energetic budgeting on the energy available for reproduction (CHAPTER III).

The metabolic energy demand in embryos and pre-feeding larvae is highly dependent on environmental factors. Decreasing concentrations of lipids and proteins were stored in eggs of European pilchard *Sardina pilchardus* (Walbaum, 1792) as the spawning season progressed, implying that eggs of higher quality are spawned at the beginning of the spawning season at low winter temperatures, when developmental times are long and food for larvae is scarce (Riveiro et al. 2000, 2004). It has been shown that *S. sprattus* is able to adjust its reproductive tactic, investing invariable amounts of gross energy into reproduction, albeit altering fecundity and oocyte quality (oocyte dry weight and lipid content) depending on environmental parameters (Döring et al. 2018a). We observed the same pattern in *E. fimbriata*: Spawning batch energy content (*SBEC*) remained temporally unchanged due to the observed trade-off between fecundity and oocyte energy content, and only spatial differences in *SBEC* could be observed (Figure 4.3). This evidences not only a generally higher productivity of females reproducing in the Saloum River's middle reaches but also that oocyte properties are tailored to the needs of early life stages as an adaptive response to hydrological settings, thereby enhancing larval growth and survival (Castro et al. 2009, CHAPTER III).

Panfili et al. (2004) did not observe salinity dependent age at length differences in *E. fimbriata*. Females sampled for this thesis, hence, were most likely of equal age at a given length regardless of sampling location. Just like for oocyte volume we observed no effect of spawner age/length on oocyte weight, composition (lipid and protein content), or energy content. Comparable observations were made for *S. sprattus* in the brackish Baltic sea (Döring et al. 2018a). The notion that oocyte quality is substantially higher in larger fish and that this leads to a higher contribution to the stock reproductive potential by older year classes despite their lower numbers (Trippel et al. 1997), seems invalid for small tropical clupeid fishes occupying stressful habitats. This conception, however, opposes a currently widely excepted paradigm that older, and thus bigger females produce more offspring of higher quality (BOFFFF: big old fat fecund female fish) (Berkeley

et al. 2004, Wiegand et al. 2007, Hixon et al. 2014). Lowerre-Barbieri et al. (2015) observed that in female South Atlantic red snapper *Lutjanus campechanus* (Poey, 1860) oocyte dry weight as a measure of egg quality was insignificantly linked with female age. A study by Allen et al. (2018) showed that older, bigger, and better-conditioned female snapper *Chrysophrys auratus* (Forster, 1801) did not produce eggs with higher lipid (triglyceride) concentrations. Both studies, therefore, failed to assess a BOFFFF effect. Ergo, in ephemeral fishes exhibiting a so-called income breeding strategy, oocyte quality seems to be directly coupled with food availability and/or prevailing hydrographic conditions, and as pointed out earlier, with specialized reproductive tactics shaped by energetic budgeting (Döring et al. 2018a). This ultimately leads to a smaller contribution of older females to the reproductive potential of a fished stock than previously thought and has implications for management strategies.

4.4. Reproductive Potential

Spawning stock biomass exhibits a limited capacity to predict recruitment (Kell et al. 2016). More promising might be the determination of physiological strength in reproduction products to estimate the survival potential of a stock (Marshall et al. 2000, 2003, Frank & Brickman 2001). Not only oocyte protein and lipid fractions change during the spawning season, also the composition of lipid sub groups varies (Green 2008). Whereas the quantity of endogenous lipid reserves in the larvae's yolk dictates the time frame in which pre-feeding larvae are able to survive, the egg's fatty acid (FA) composition alter larval development and growth (Grote et al. 2011). In clupeoids, the FA composition of the muscle and gonads resembles precisely the one of the plankton, indicating a rapid transfer of FA composition from prey to fish tissues (Shirai et al. 2002, Castro et al. 2010, Fuiman & Faulk 2013). Changes in water temperatures, salinity, and upwelling intensity caused by climate change are suspected to have direct effects on community structures and FA composition in phytoplanktonic food items relevant for clupeids (Bode et al. 2009, Demarcq 2009, Nche-Fambo et al. 2015, Hixson & Arts 2016).

Especially under high temperature and elevated salinity conditions hatching success of marine fish eggs and survival probabilities of early life stages are prone to impairment (Hempel 1979, Martin 1988, Shi et al. 2008). The nutritional quality of oocytes, in particular in terms of their FA composition, is, therefore, an important factor during the ontogeny and embryonic development of clupeid fishes in hypoosmotic environments (Tocher & Sargent 1984, March 1993, Tocher 2010). Particularly essential fatty acids (EFA) affect osmoregulation capabilities in marine fish early life stages (Koven et al. 2003, Willey et al. 2003, Bransden et al. 2004), and deficiencies can be expected to cause elevated offspring mortality in hypersaline environments, ultimately modifying a stock's reproductive potential.

In *E. fimbriata*, oocyte EFA fractions were positively correlated to the female's reproductive investment (batch fecundity, gonado-somatic index, oocyte dry weight) and linked to ambient temperature and salinity conditions (CHAPTER II, Figure 4.4). These relationships could partially be explained through the species' selective feeding behaviour (Lazzaro 1987): preying on diatoms with beneficial FA profiles due to physiological modifications (homeoviscous adaptation) caused by elevated water temperatures inside the estuary (Guschina and Harwood 2006; Hixson and Arts 2016; CHAPTER II). This adaptive advantage may be part of the reason why clupeid larvae exhibit a particularly high resilience to salinity (up to 55) in the Sine Saloum estuary (Sloterdijk et al. 2017). Even though oocytes sampled inside the Saloum River from July to October (rainy season) exhibited the lowest fractions of the storage lipid 18:1 (n-9) and the lowest energy contents, female reproductive potential can be assumed to be highest during this time of year (CHAPTER II, CHAPTER III). This is because during the rainy season females exhibited the highest batch fecundities and oocyte EFA fractions (CHAPTER II).

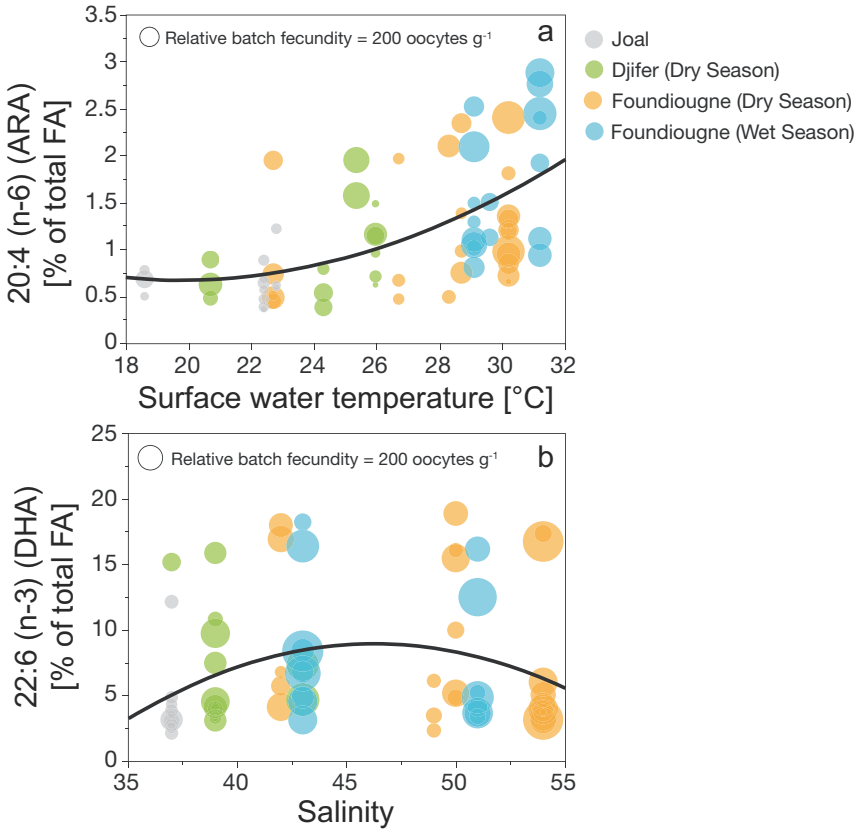

Figure 4.4 Relationships of the investigated essential fatty acids 20:4 (n-6) and 22:6 (n-3) with (a) surface water temperature and (b) surface water salinity. Respective bubble sizes display the relative batch fecundity of individual female *Ethmalosa fimbriata*. Different colors indicate the sampling location and season. ARA: arachidonic acid; DHA: docosahexaenoic acid; FA: Fatty acids

During this time of year, water temperatures were high and salinities were less stressful due to freshwater input, potentially allowing for rapid egg and larval development (Holliday 1969, Jatteau et al. 2017, Döring et al. 2018b). These findings, however, partly contradict the previously addressed paradigm that oocyte quality is negatively correlated with batch fecundity in teleost fishes. Our results also highlight that egg quality assessment is complex and interpretations are only possible in the light of the prevalent hydrographic conditions.

4.5. Stock Structure

Species may react to environmental stressors through adaptation by genome evolution, which ultimately results in genetic changes (Hoffmann & Willi 2008, Hoffmann & Sgró 2011). *E. fimbriata*'s Senegalese and Gambian stock is thought to be one population with an age-specific habitat use (Charles-Dominique & Albaret 2003). It was previously suggested that in Senegal, reproduction occurs inside the estuaries as well as at sea. Juveniles are suspected to use the estuary as their main nursery ground (Gning et al. 2007), and large adults emigrate to the sea later on (Charles-Dominique & Albaret 2003). This hypothesis, however, could not be supported by our observations on female length frequency distributions throughout the study area (CHAPTER III). Durand et al. (2013) identified two distinct genetic clusters in *E. fimbriata*, one occupying West African coastal waters (Guinea, Banc d'Arguin in Mauritania) and another estuarine environments (Sine Saloum delta, Gambia, and Casamance Rivers). In past studies, no individuals from Senegalese shelf waters were sampled for genetic analyses, leaving room that the southern Senegalese neritic and estuarine populations are genetically indistinguishable. It may also be possible that there is a strictly coastal population, inhabiting the shelf waters from Mauritania until at least Guinea, and a strictly estuarine one, inhabiting the Sine Saloum, the Gambia, and the Casamance River (Panfili et al. 2004, Durand et al. 2013). Differences in *E. fimbriata* oocyte EFA profiles may as well lead to the assumption that there are genetic differences between the marine and the estuarine population (CHAPTER II). In cod, for instance, individuals from different source population continued to spawn eggs with different EFA signatures when fed the same diet (Pickova & Dutta 1997).

We identified significant spatial differences in reproductive investment and otolith fingerprints in female *E. fimbriata* (Figure 4.5, CHAPTER III). This may not only evidence several stock spawning components in southern Senegalese waters, which are characterized by delimited home ranges and distinctive productivity. It might also be possible that differences in the examined reproductive traits (home range, oocyte calorific value, *SBEC*) may be subject to genetic variations, substantiating the suspicion that there are genetically distinct populations in Senegalese coastal waters. Comparably, there appear to be genetically distinct *S. sprattus* populations inside the Baltic sea (Limborg et al. 2009). The species' migration behaviour, however, leads to an extensive

mixing of different stock components and clear separation of sprat sub-populations proves to be challenging (Aro 1989). Thus, observed spatial differences in sprat fecundity, oocyte dry weight, spawning batch energy content, and oocyte EFA composition may be either attributed to adaptations within the realm of phenotypic plasticity or to genetic alterations (Döring et al. 2018a).

In migratory clupeid fish species, genetic mixing may not allow for a clear-cut separation between stock components. Our approach of combining spawning energetics and otolith elemental fingerprints may therefore be a valuable method for delineation of stock components in clupeid fishes occupying diverse spawning habitats.

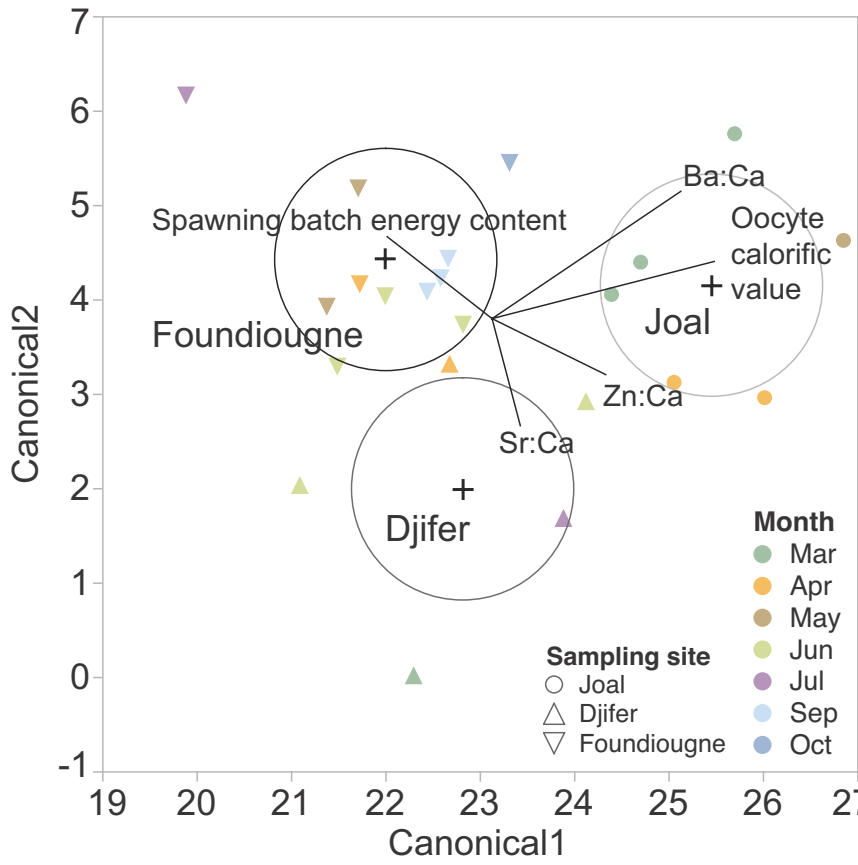

Figure 4.5 Linear discriminant function analysis employing spawning energetics and otolith element:Ca ratios in female *Ethmalosa fimbriata* sampled at the Senegalese South Coast (Joal), at the Saloum River's mouth (Djifer), and inside the river's middle reaches (Foundiougne) from March to October 2014. Ellipsoids are of equal sizes and include ca. 50% of a sampling site's data points

4.6. The Reproductive Tactics of *Ethmalosa fimbriata*

A species' "reproductive tactic" is the extent to which specific reproductive traits are expressed in a specific environmental situation (Wootton 1998, Green 2008, Jakobsen et al. 2009). In this thesis, several reproductive traits of *E. fimbriata* have been investigated with special attention on environmental conditions at the Senegalese South Coast and inside the Saloum estuary. The energy invested in reproduction by individual females remained unchanged during the spawning period and did not differ between female size classes, indicating no significant effect of energetic budgeting on reproductive investment. In southern Senegalese waters, *E. fimbriata*'s stock comprises oceanic and estuarine spawning components, each employing distinct reproductive tactics.

Spawning times and temporally unaltered reproductive investment/potential hint towards favourable hydrographic condition for offspring survival at the Senegalese South Coast after the onset of upwelling at the beginning of the year (February - May). Spawning during the upwelling season is a common feature in clupeids reproducing in the Canary Current upwelling system (Mbaye et al. 2015, Tiedemann et al. 2017). In Senegalese shelf waters, rather the place and timing of spawning than the fine-tuning of reproductive traits seem to be decisive for recruitment success.

The stock's spawning component reproducing at the Saloum River's mouth exhibits a more variable, albeit temporally limited spawning tactic. To take advantage of relatively low salinities and favourable water temperatures, reproduction at the Sine Saloum's mouth during the dry season seems to be common in clupeid fishes inhabiting the system (Sloterdijk et al. 2017). Inside the Saloum River's middle reaches, *E. fimbriata* spawned more eggs but of lower energy content during the warm wet season and vice versa during the rather cold dry season. Higher energy reserves in eggs spawned at lower water temperatures and at high salinities sustain elongated ontogenetic times due to lowered metabolism (Alderdice & Forrester 1968, Alderdice & Velsen 1971, Jatteau et al. 2017). Female reproductive potential can be determined to be highest inside the Saloum River's middle reaches during the wet season. This is because fecundity and oocyte EFA fractions were superior to the ones of females sampled throughout the Saloum River during the dry season. In the hypersaline Saloum River's middle reaches, *E. fimbriata*, thus, adapted a spawning tactic that aims at intensifying spawning during the wet season, when water temperatures are high and the system's salinity is less stressful.

Considering the prolonged reproductive period, properly timed peak spawning, overall higher reproductive investment, and enhanced reproductive potential because of beneficial oocyte EFA fractions, the productivity of the estuarine spawning components has to be assumed as significantly higher than the coastal ones.

4.7. Advice for Management and Outlook

Preserving larger fish is often advocated as a conservation measure to help fish stocks buffer environmental variation and fishing pressure. The general consensus is that length- and age-dependent reproductive traits confer a higher reproductive value to females in higher size classes (Le Bris et al. 2015). Our observations indicate no positive relationship between female size and reproductive value, rendering gear restrictions to reprieve larger fish unsuitable. Taking into account the observed reproductive tactics, time and area closures may be more promising to conserve the stock's reproductive potential and to warrant sustainability (Sadovy & Cheung 2003, Sadovy et al. 2003). In the case of *E. fimbriata*'s stock in southern Senegalese waters, this would imply halted fishing activities at the coast from February to May, at the estuary's mouth from April to June, and inside the system's middle reaches from September to November. These timeframes are, however, variable and dependent on hydrological and meteorological processes, which are themselves subject to annual alternations.

Berkeley et al. (2004b) hypothesised that in any given year, only a small proportion of female spawners is responsible for nearly all successful recruitment, and that this success arises from reproductive activities matching those geographical areas that happen to have hydrographical conditions favourable for larval survival (Hedgecock hypothesis) (Jakobsen et al. 2009, Hedgecock & Pudovkin 2011). Additionally, less productive stock components, that is to say, *E. fimbriata*'s coastal sub-population, are more likely to become overexploited and a decline in catch rates could ultimately compromise the fisheries' activity and affect the depending socio-economic sector (Jennings et al. 1998). Successful stock management should, therefore, include the objective of maintaining reproductive potential over the full geographic range of the species (Berkeley et al. 2004).

An ecosystem approach to fisheries not only takes into account the impact of fisheries on the ecosystem but also the ecosystem's influence on fisheries. The ecosystem impacts a fishery in several ways, among others, modifying the reproductive potential of target species. Thus, any factor altering species' habitat characteristics has concomitant effects on reproductive success (Jakobsen et al. 2009). This thesis goes through great length to emphasize that maternal and environmental effects on recruitment play a significant role and must be recognized in assessment efforts for a more effective stock management.

E. fimbriata is so far likely to benefit from the severe impacts of climate change on its spawning habitat because of a complex stock structure and by employing auspicious reproductive tactics. The species may therefore outcompete other pelagic fish species with lower adaptive potentials. High plasticity in reproductive traits combined with high fecundities and small

generation times in clupeid fishes such as *E. fimbriata* may lead to an enhanced fitness under rapid environmental changes (Reed et al. 2011). A further decrease in precipitation and a concerted elevation of ambient salinities in the Sine Saloum estuary will have negative impacts on *E. fimbriata*'s reproductive potential, on the carrying capacity of its habitat, and ultimately on its stock size. In future temperature regimes, however, poleward expansion of the species' distribution range seems also probable, given the high adaptability of clupeids (Sabatés et al. 2006).

CHAPTER I

5. *ETHMALOSA FIMBRIATA* (BOWDICH, 1825), A CLUPEID FISH THAT EXHIBITS ELEVATED BATCH FECUNDITY IN HYPERSALINE WATERS

Julian Döring, Maik Tiedemann, Moritz Stäbler, Hans Sloterdijk, and Werner Ekau

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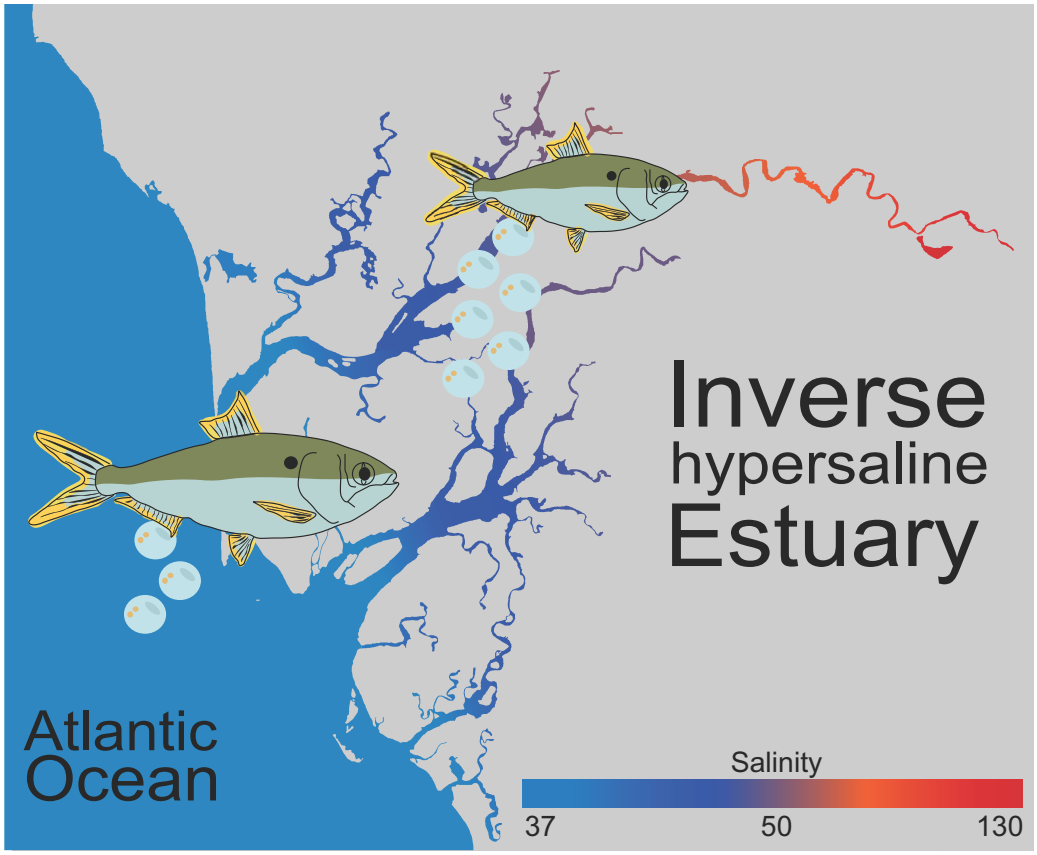
Inverse
hypersaline
Estuary
Atlantic
Ocean
Salinity
37
50
130

Abstract

Little is known about the concerted influence of temperature and salinity on the fecundity of clupeid fishes. Due to a globally changing climate, both physical parameters might act as stressors, severely affecting the reproductive potential of clupeid fish populations inhabiting tropical estuaries. Differences in relative batch fecundities, the gonado-somatic index, and the condition index of bonga shad *Ethmalosa fimbriata* were analysed in individual females sampled at the Senegalese coast and inside the inverse Sine Saloum estuary, where salinity increases upstream in all seasons. Multiple linear regression models on fecundity and gonadal energy storage show that clupeids can adapt towards increasing their reproductive effort at temperatures (26 - 30°C) and salinities (42 - 51), which by far exceed marine conditions, in an effort to maximize recruitment success. This spawning tactic, however, was accompanied by a trade-off between reproductive investment and somatic growth, which ultimately limits the species' reproductive investment inside the estuary. The observed high variability in batch fecundities might be a viable mechanism to adjust to fluctuating and rather extreme environmental conditions. Understanding the spawning biology of exploited clupeid fishes in drastically changing environments is crucial for evaluating the reproductive potential of stocks at the outer reach of their physiological performance curve.

Keywords: Climate change · Senegal · Inverse estuary · Reproductive potential · Temperature · Salinity · Spawning · Fecundity · Condition · Trade-off

5.1. Introduction

While spawning traits of marine fish species in marine, brackish, or fresh waters are extensively studied (Kucera et al. 2002, Nissling et al. 2006), little is known about reproductive characteristics of fishes under hypersaline conditions, exposed to salinities >40. Such extreme conditions require elevated maintenance costs for osmoregulation and are especially challenging for the mature female (Kirschner 1993, Sangiao-Alvarellos et al. 2003), as well as for its eggs and larvae (Holliday & Blaxter 1960, Jia et al. 2009). Spawning as such is metabolically one of the most demanding activities in the lifetime of female fishes (Leggett & Carscadden 1978, McBride et al. 2015), making reproduction under hypersaline conditions an energetically very expensive endeavour (Panfili et al. 2006).

Adaptive phenotypic plasticity is a valuable mechanism for populations to deal with rapid environmental fluctuations such as changes in salinity (Aykanat et al. 2011). Clupeids are generally able to cope with extreme environmental conditions by varying their life history traits via either genetic changes or phenotypic plasticity (Stearns 1992, Rogers & Ward 2007). For example, Baltic sprat Sprattus sprattus balticus (Schneider 1908) exhibit a decrease in batch fecundities along a declining salinity gradient within the brackish Baltic Sea (Alheit 1988, Müller et al. 1990, Alekseev & Alekseeva 2005, Haslob et al. 2011, Döring et al. 2018a).

Temperature not only has significant effects on metabolic rates and fecundities in clupeid fishes, but also on egg and larval development (Tanasichuk & Ware 1987, Milton et al. 1995, Leonard et al. 1999). Under conditions of extreme temperature and salinity, adult survival rates of marine fishes, as well as egg and larval development are impaired (Hempel 1979, Martin 1988). At the boundary of its physiological performance curve an adult fish is thus forced to budget between survival and reproductive capacity (Pörtner et al. 2010). Therefore, in a heterogeneous environment a species' reproductive potential may exhibit strong variations spatially as well as temporally (Milton et al. 1995, Peck et al. 2012).

Little is known about the concerted influence of temperature and salinity on an individual's condition, growth, and consequently on the amount of eggs it may produce (Panfili et al. 2004, Tseng & Hwang 2008, Haslob et al. 2011). Water temperature has already been identified as a factor influencing fecundity in marine fishes. The influence can be either passive through its regulatory function of phytoplankton abundances and thus food availability or active through altering individual's metabolic rate (Woodhead 1960, Hempel 1971, McBride et al. 2015). In mature fish, feeding conditions directly affect the individual's condition and the amount of energy that can be put into reproduction (Marshall & Frank 1999, Somarakis et al. 2012, McBride et al. 2015). Furthermore, osmoregulation processes are energetically expensive

and may therefore interfere with species' reproductive traits at elevated salinities (De Vlaming 1971, Sangiao-Alvarellos et al. 2003). All of these variables potentially affect subsequent recruitment success and have to be considered when evaluating a species' reproductive potential at the boundary of its physiological optimum range (Morgan 2008, McBride et al. 2015).

A variety of studies lead to the conclusion that the bonga shad *Ethmalosa fimbriata* (Bowdich, 1825), is locally adapted to cope with a wide range of environmental conditions in terms of its morphology, growth, and reproductive traits (Bainbridge 1957, 1961, 1963, Facade & Olaniyan 1972, Panfili et al. 2004, Panfili et al. 2006). The species is distributed in West African Atlantic waters from Mauritania to Angola (Lozano-Ray 1950, Paugy et al. 2003). Here, *E. fimbriata* spawns either in the sea (Bainbridge 1961), or in estuaries and lagoons (Facade & Olaniyan 1972). A physiology adapted to euryhaline conditions allows the species to cope with varying salinities in these diverse habitats granting it to tolerate salinities from around 0 to 97 (Panfili et al. 2006) and even to reproduce in waters with salinities up to 66 (Charles-Dominique & Albaret 2003). It is one of the most important fishes targeted by artisanal fisheries in Cameroon, Ivory Coast, Nigeria, and Senegal (Albaret & Charles-Dominique 1982, Deme et al. 2001, Moses et al. 2002).

Especially in southern Senegalese coastal waters *E. fimbriata* has to endure a wide range of varying environmental settings. Former studies demonstrated that the species spawns all year round in the lower and middle reaches of the Saloum River (Scheffers et al. 1972, Albaret & Gerlotto 1976), an inverse hypersaline environment with monthly fluctuations in temperature and salinity (Pagès & Citeau 1990). The rather extreme environmental conditions in the Sine Saloum estuary were used to investigate the reproductive responses of a clupeid fish at the outer reach of its physiological performance curve. Due to a globally changing climate the inversion of estuaries in other parts of the dry tropics can be expected in the near future (Wolanski 1986, Pagès & Citeau 1990, Ridd & Stieglitz 2002). Thus, understanding the spawning biology and adaptation potential of commercially important fish species is the key to predict inevitable impacts on the ecosystems and local fisheries.

The current study focused on how this hypersaline environment influences the spawning biology of female *E. fimbriata* and how the species is adapted to cope with the aforementioned extreme salinity conditions. More specific, it was attempted to reveal possible energetic trade-offs accompanied by inhabiting and spawning in hypersaline environments. Consequently, variations in somatic condition, gonado-somatic index, as well as in batch fecundity of female *E. fimbriata* were investigated along a spatial and temporal salinity and temperature gradient at the Senegalese coast and within the hypersaline Saloum estuary.

5.2. Materials & Methods

5.2.1. Study area

Located 100 km south of Dakar (between 13°55' and 14°10' N, and 16°03' and 16°50' W), the Sine Saloum delta is comprised of three main branches, the Saloum, the Diomboss, and the Bandiala (Figure 5.1). An extended dry season is characteristic for this region, cool from November to March, and warm from April to June. The wet and warm season is rather short, lasting from July to October.

Total rainfall has been steadily decreasing since the 1920's, with a particularly severe decline since 1961. Combined effects of tides, reduced river run-off, intense evaporation, and shallow waters in the estuary's mouth have led to high salinity and overall inversion of the salinity gradient (Pagès & Citeau 1990). Consequently, salinity increases upstream in all seasons in the Saloum River. In the river's upper reaches, surface water temperatures thus exceed 30°C and salinity levels are 2 to 3 times higher than those of seawater by the end of the dry season (Simier et al. 2004).

Sampling took place at the Senegalese coast and inside the Saloum River from February to October 2014, during the peak of *Ethmalosa fimbriata*'s spawning season (Charles-Dominique 1982, Panfili et al. 2004). Three different sites were sampled: Joal (Senegalese South Coast, 14°9.1' N; 16°51.7' W), Djifer (Saloum River's mouth, 13°57.8' N; 16°44.8' W), and Foundiougne (upstream Saloum River, 14°8.1' N; 16°28.1' W) (Figure 5.1).

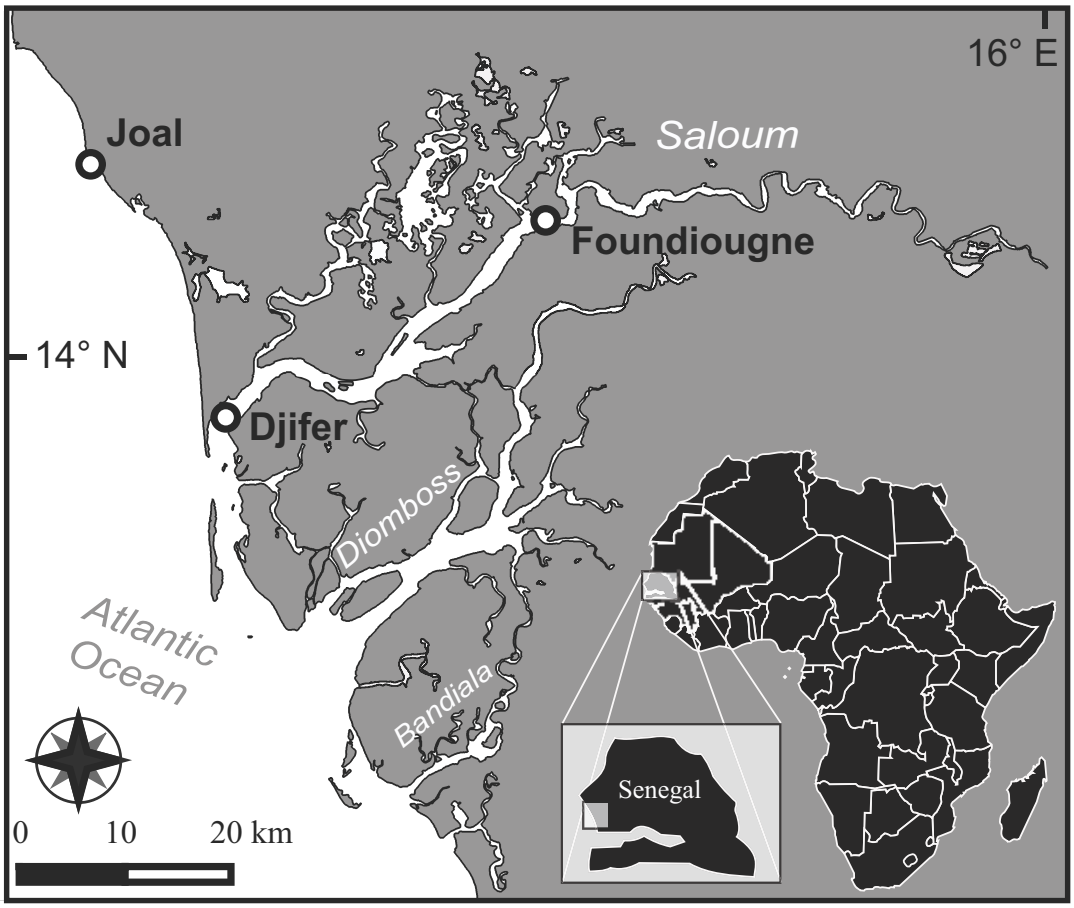

Figure 5.1 Map of the Senegalese coast and the Sine-Saloum estuary including sampling sites: Joal, Djifer, and Foundiougne

5.2.2. Environmental parameters

To get an overview on seasonal changes of environmental conditions, water temperatures, and salinity were measured. Because the upwelling area in front of Senegal (mean depth <20 m) and the Saloum River (mean depth <10 m) are well mixed water bodies, surface water temperatures and salinities were employed (Saos & Pagés 1985, Sloterdijk et al. 2017, Tiedemann & Brehmer 2017). MODIS satellite-derived (Aqua, level 2, 0.1 degrees) sea surface temperatures (https://oceancolor.gsfc.nasa.gov/) were assessed at Joal (20 km radius) and Djifer once per sampling week to enhance spatial coverage by avoiding cloud cover. As no remote sensing data for inland waters were available, the Saloum River's surface water temperatures were recorded in situ once per sampling week with a digital thermometer (ama-digit ad 15th; precision 0.4%; accuracy 0.4%). For all stations salinity was measured with a handheld refractometer (Aqua Medic; precision 0.7%, accuracy 0.2%) using a Practical Salinity Scale (PSS-78). Monthly means in MODIS satellite-derived (Aquarius, level 3, 0.5 degrees) sea surface salinities (https://oceancolor.gsfc.nasa.gov/) were used to assess the coastal ocean's salinity in a radius of 20 km around Joal.

5.2.3. Fish sampling

Fish were caught with gill nets (32 - 36 mm mesh size) by local fishermen, and immediately stored on crushed ice after landing. To ascertain the species' batch fecundity, ca. 1000 fish per sampling site and month were examined in order to find stage V females. Staging of fish was conducted macroscopically after a key by Blay Jr. & Eyeson (1982). In order to discriminate between mature females with ovaries containing fully hydrated oocytes (stage V) and females that have recently spawned (spent, stage VI) further descriptions on maturity stages in clupeid fishes were consulted (West 1990, ter Hofstede et al. 2007). Running ripe females were not sampled, since they might have released part of the egg batch. Overall, the ovaries of 189 specimens were dissected and transferred to a 4% borax buffered formaldehyde and freshwater liquid.

Fish were measured by fork length to avoid falsifications due to fin damage. Accordingly, 100 fish with intact caudal fins were measured twice, by fork length as well as total length, to obtain a length coefficient for subsequent length data transformation. Conversions of fish fork length (L_F) to total length (L_T) were conducted using the equation $L_T = 1.0007 \times L_F + 3.9$ (Student's t-test, $p < 0.05$, $r^2 = 0.83$).

5.2.4. Somatic and gonadal energy storage

In order to access the nutritional status of immature female fish (Stage I - IV), a condition index (K) (Equation 5.1) was calculated for each individual using wet weight (W_W, ±0.1 g), total length (L_T, nearest mm) (Lloret et al. 2014), and b of the length-weight relationship. The condition index (K_{hyd}) (Equation 5.2) and gonado-somatic index (I_G) (Equation 5.3) of hydrated female spawners (Stage V) was based on the ovary-free body weight (W_{OF}, ±0.1 g) and the ovary weight (W_{Ov}, ±0.0001 g) (Zydlewski et al. 2003). These indices were used as proxies for the somatic and gonadal energy storage of female spawners.

$$K = \frac{W_W}{L_T^{\,b}} \times 1000 \tag{5.1}$$

$$K_{hyd} = \frac{W_{OF}}{L_T^{\,b}} \times 1000 \tag{5.2}$$

$$I_G = \frac{W_{Ov}}{W_{OF}} \times 100 \tag{5.3}$$

5.2.5. Fecundity

E. fimbriata is an indeterminate batch spawner (Albaret & Gerlotto 1976). Therefore, measuring the number of hydrated oocytes produced in a single spawning batch is the only useful method to determine fecundity (Hunter et al. 1985). Absolute batch fecundity (*ABF*) was consequently assessed gravimetrically using the hydrated oocyte method for indeterminate spawners (Alheit 1993, Olney & McBride 2003, Haslob et al. 2011). *ABF* was divided by W_{OF} to calculate the relative batch fecundity (*RBF*) (Alheit 1988). Due to high variability in *E. fimbriata*'s batch fecundities, we re-analysed the 10% of the sampled ovaries containing the least amount of oocytes. We measured the diameters of seemingly hydrated oocytes within the questionable samples and compared these values with diameter values of hydrated oocytes stored in ovaries with high absolute batch fecundities, which were sampled in the same months, using ANOVA (analysis of variance) techniques. Ovaries containing oocytes with significantly lower diameters were rejected. Only hydrated oocytes with a diameter greater than 0.80 mm were taken into account for fecundity analysis. Additionally, ovaries containing oocytes without significant differences in diameter were re-examined macroscopically.

5.2.6. Statistical analyses

The relationships between L_T and W_W were described using power functions. Data on L_T and W_W were log-transformed in order to test for spatial differences in the resulting linear growth models. The assumption of parallel lines was tested and intercepts were subsequently compared using analysis of covariance (ANCOVA) (McDonald 2009). The parameters of the linear regressions for L_T and *ABF*, as well as for W_{OF} and *ABF*, were also compared using ANCOVA. Regression coefficients for the relationships between *FL* and L_T, L_T and W_W, were tested for significance using Student's t-tests. Differences in *K* were tested using ANOVA with both sampling month as well as sampling location as independent variables. Additionally, for each sampling location, differences in K_{hyd}, I_G, and *RBF* were tested applying ANOVA with sampling month as independent variable. Given the fact that ANOVA techniques are quite robust against small sampling sizes if the assumption of homogeneity of variances is met (Brown & Forsythe 1974), data on I_G and *RBF* were square root transformed (Schmider et al. 2010, Haslob et al. 2011). In cases where significant differences were detected, post hoc multiple comparisons were performed. The employed Tukey HSD (honest significant difference) test calculates p-values that have been corrected for the number of independent pair-wise comparisons that are possible given the number of factor levels. Statistical analyses were carried out using JMP 11.0.1 (SAS Institute Inc., Cary, NC, http://www.jmp.com).

Since tolerance curves describing physiological processes are a common tool in ecology, predictive models were used, which allowed a comparison between *E. fimbriata*'s spawning preferences and condition optimum (Huey &

Stevenson 1979, Cury & Roy 1989, Pörtner & Peck 2010). After interactions between independent variables were tested, we employed multiple linear regressions to reveal the respective responses of *RBF*, I_G, and K_{hyd} to temperature (T, T^2) and salinity (S, S^2). The resulting minimal adequate models were tested for the constancy of variances (residuals against fitted values; left hand side plots in Appendix A) and normal distribution of their residuals (normal quantile-quantile plot; right hand side plots in Appendix A; and Anderson-Darling tests and Cramer-von-Mises tests with a significance threshold of $p = 0.1$). Where any of these two assumptions were violated, we transformed the response variable and re-ran the multiple regression. This was the case for all three dependent variables and thus square-root transformations were applied. All final minimal adequate models show constancy of variance and normal distribution of their residuals (Appendix A). Models were built in RStudio: Integrated Development for R 1.0.44 (RStudio, Inc., Boston, MA, www.rstudio.com).

5.3. Results

5.3.1. Environmental conditions

Night-time surface water temperatures at the three sampling sites steadily increased throughout the sampling period, peaking in October. Surface water temperatures were generally higher inside at Foundiougne than at Joal, with intermediate values recorded at Djifer. At the beginning of the sampling period (February), salinity was determined to be 39 at Foundiougne as well as at Djifer. In the following months, salinity increased steeply at Foundiougne, eventually peaking in July at 54 until it dropped to 43 in September and October. At Djifer, salinity peaked at 43 in July and August before dropping down to 32 in September. The tropical open ocean's salinity at Joal negligibly fluctuated around 37 throughout the entire sampling period (Figure 5.2).

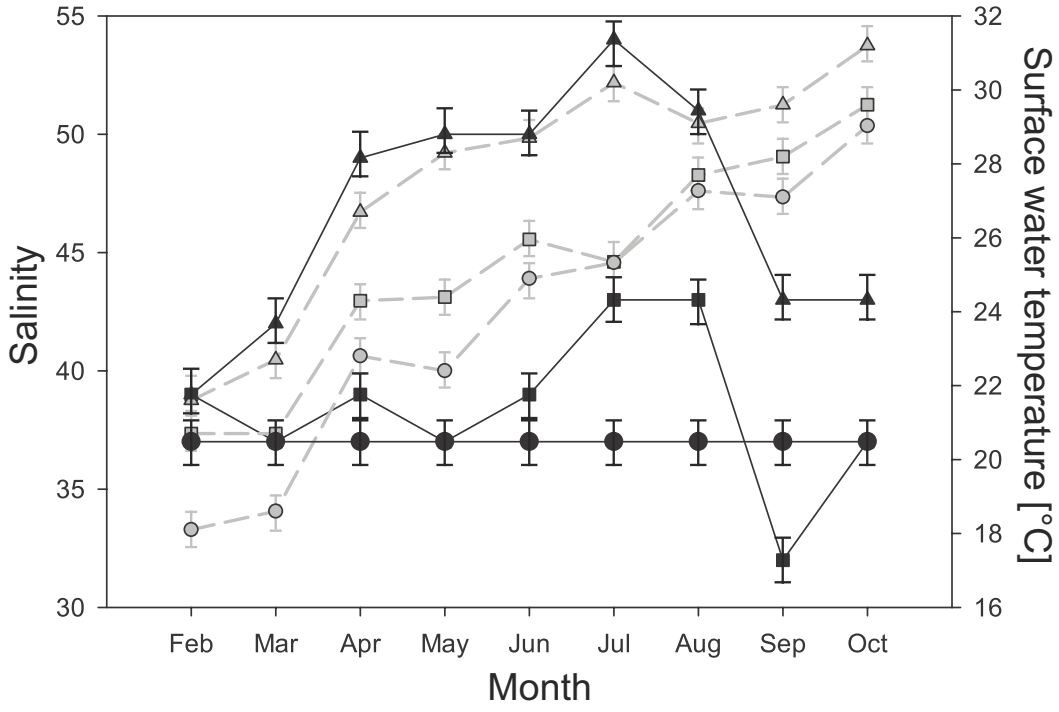

Figure 5.2 Night-time surface water temperatures (± Standard Error (SE); grey) and surface water salinities (± SE; black) of the Senegalese South Coast and the Saloum River as recorded at Joal (circles), Djifer (squares), and Foundiougne (triangles) throughout the sampling period

Table 5.1 Monthly sampling sizes for immature (stage I-IV) and hydrated (stage V) female *Ethmalosa fimbriata* at the three sampling sites: Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches).

Sampling site	Sampling month	Stage I - IV	Stage V
Joal	February		
	March	30	8
	April	24	3
	May	44	20
	June	35	
	July	42	
	August	26	
	September	34	
	October	29	
Djifer	February	16	8
	March	57	2
	April	19	25
	May	38	
	June	32	7
	July	37	4
	August	37	
	September	24	
	October	27	
Foundiougne	February	52	2
	March	20	15
	April	66	4
	May	44	2
	June	40	5
	July	26	23
	August	42	29
	September	51	3
	October	27	29

5.3.2. Length-weight relationship

A total of 1108 female *E. fimbriata* were sampled for this study, 919 specimens were identified as stage I-IV, the ovaries of 189 individuals stored hydrated oocytes (Table 5.1). Different power functions were calculated to describe the length-weight-relationships of females sampled at the three sampling sites (Figure 5.3). Comparison of linear growth models revealed that females sampled at Foundiougne weighed significantly less at a certain length than their counterparts sampled at Djifer and Joal (ANCOVA, $F_{(3, 1107)} = 3837.10$, $p < 0.0001$; Tukey HSD, $p < 0.001$). The length-weight relationship of all sampled females across all sampling sites could be described by the following function (Equation 5.4, Student's t-test, $p < 0.05$, $r^2 = 0.90$, $n = 1108$):

$$W_W = 0.0015 \times {L_T}^{3.62} \tag{5.4}$$

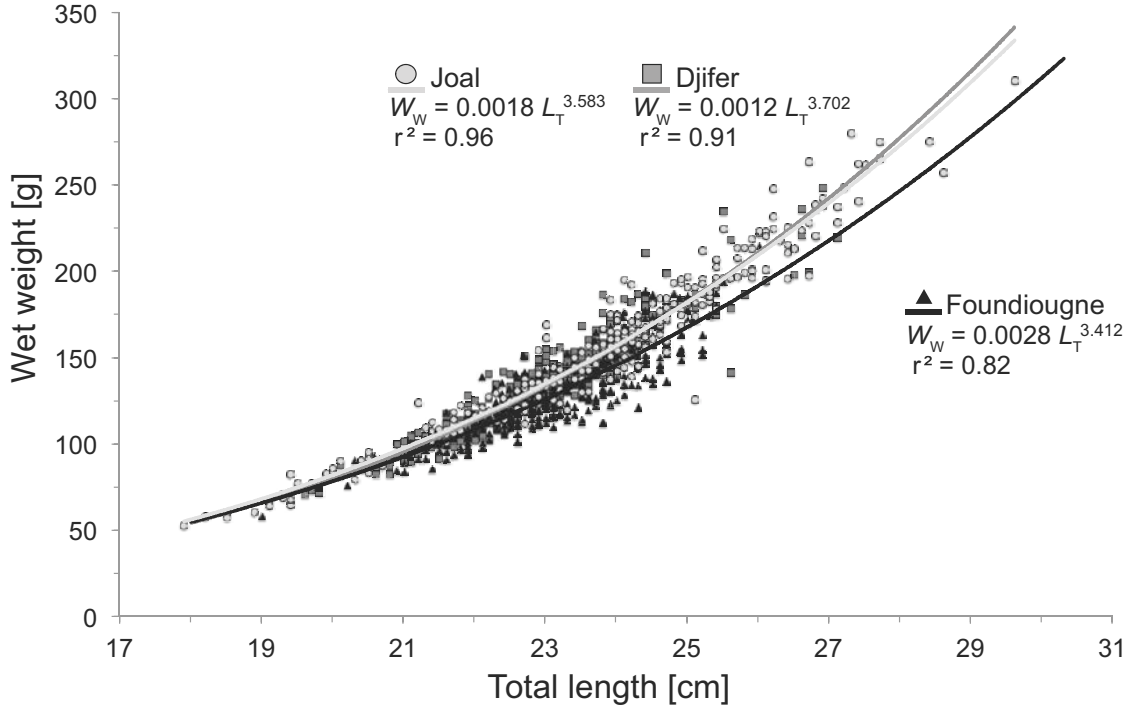

Figure 5.3 Length-weight-relationships for all female *Ethmalosa fimbriata* sampled at Joal (coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches)

5.3.3. Somatic and gonadal energy storage

A statistical analysis revealed not only significant monthly differences in *K* for each station but also significant differences between stations for each month apart from February. At Djifer, condition of female fish (stage I - IV) peaked in February as well as in July (ANOVA, $F_{(8, 286)} = 7.50$, $p < 0.0001$; Tukey HSD, $p < 0.05$). Comparably, mean *K* of females sampled at Foundiougne was significantly higher in February and in July than in most other months, except in March and June (ANOVA, $F_{(8, 367)} = 10.92$, $p < 0.0001$; Tukey HSD, $p < 0.05$). Females were in a significantly better condition at Djifer than at Foundiougne in all months except for October (Tukey HSD, $p < 0.05$). Mean *K* of females sampled at Joal was significantly higher than the one of individuals sampled at Foundiougne in all months except for March, April, and July (Tukey HSD, $p < 0.05$). Still, mean condition of immature females was always lowest in specimens sampled inside the Saloum River's middle reaches at Foundiougne (Figure 5.4).

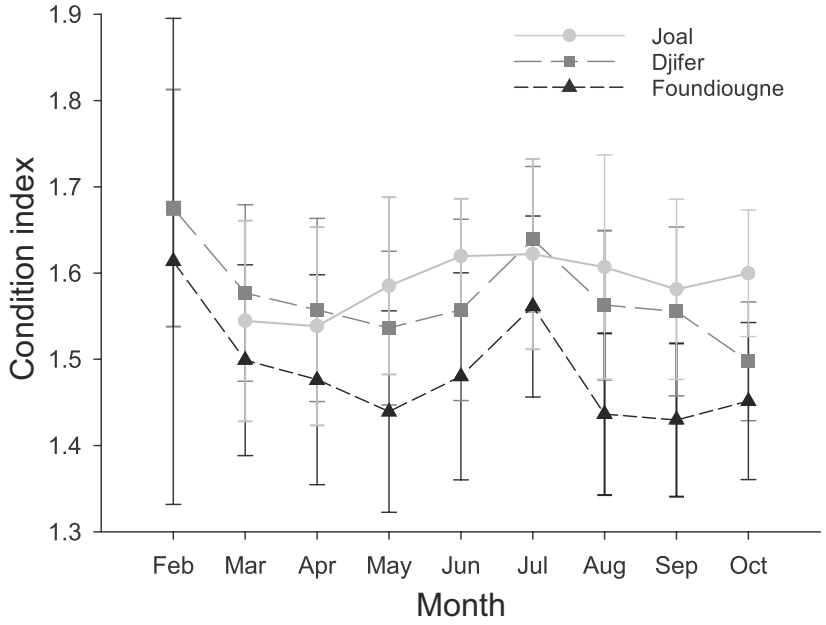

Figure 5.4 Mean (± Standard Deviation (SD)) monthly changes in condition index of stage I - IV female *Ethmalosa fimbriata* sampled at Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches)

The condition index (K_{hyd}) of stage V showed a decreasing trend at all sampling sites during the progression of the sampling period (February to October). Mean condition of spawners sampled at Foundiougne was significantly higher in February than in September (ANOVA, $F_{(8,\ 111)} = 3.39$, $p < 0.01$) (see Appendix Table A for Tukey HSD results). Comparably, K_{hyd} at Joal (ANOVA, $F_{(2,\ 30)} = 0.46$, $p = 0.64$) and Djifer (ANOVA, $F_{(4,\ 45)} = 1.82$, $p = 0.14$) was higher in March than in all other sampling months (Figure 5.5).

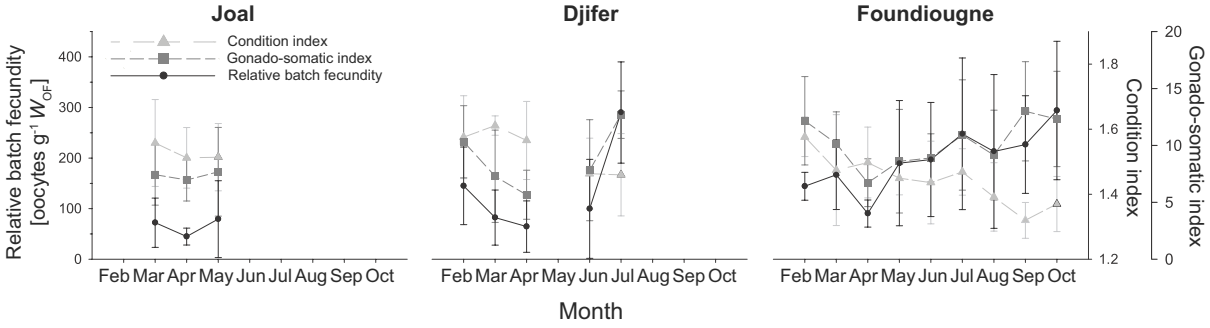

Figure 5.5 Mean (± SD) relative batch fecundity, condition index, and gonado-somatic index of stage V females per sampling site and month

At Djifer (ANOVA, $F_{(4,\ 45)} = 7.33$, $p < 0.001$) and Foundiougne (ANOVA, $F_{(8,\ 111)} = 2.05$, $p < 0.05$) mean gonado-somatic index (I_G) of stage V females reached a significant minimum in April and subsequently increased again (Appendix Table A). At Joal (ANOVA, $F_{(2,\ 30)} = 0.03$, $p = 0.98$) I_G remained constant throughout the three months in which spawning females could be sampled.

5.3.4. Fecundity

Fecund females were sampled in the size ranges (L_T) of 23.1 cm – 27.5 cm, 20.8 cm – 26.9 cm, and 19.9 cm – 28.3 cm at Joal, Djifer, and Foundiougne, respectively. The variance in number of produced oocytes per spawner was generally high throughout the three sampling sites: *ABF* at Joal ranged between 3,316 hydrated oocytes in a 25.4 cm individual, and 66,375 ripe oocytes in a 26.1 cm long female both sampled in May. At Djifer, a 23.3 cm female sampled in April exhibited the lowest *ABF* with 2,200 oocytes, while in February a similarly sized individual (23.5 cm) showed an *ABF* of 39,490 hydrated oocytes. At Foundiougne *ABF* ranged between 3,160 oocytes in a 25.5 cm female and as much as 85,410 oocytes in a 24.8 cm specimen both sampled in July. An effect of the female's size on the number of produced oocytes was controlled for by applying ANCOVA on the linear regressions between L_T and *ABF*, as well as W_{OF} and *ABF*. The assumption of parallel lines was met. The intercept of L_T and *ABF* was significantly higher in females sampled at Foundiougne and Djifer than at Joal (ANCOVA, $F_{(3,\ 188)} = 18.4923$, $p < 0.0001$, Tukey HSD, $p < 0.0001$). Also, the intercept of W_{OF} and *ABF* was significantly higher at Foundiougne than at Djifer and Joal (ANCOVA, $F_{(3,\ 188)} =$

16.85, $p < 0.0001$, Tukey HSD, $p < 0.01$). Therefore, *ABF* values were always highest at Foundiougne for the same female size.

In spite of considerable inter-individual variation, significant monthly variations in relative batch fecundity (*RBF*) could be observed. Whereas no monthly differences in mean *RBF* were observed in females sampled at Joal (ANOVA, $F_{(2, 30)} = 0.37$, $p = 0.69$), fecundity was significantly lower in February than in April at Djifer. During the consecutive months mean *RBF* increased again at this sampling site: *RBF* was determined to be significantly higher in July than in all other sampling months (ANOVA, $F_{(4, 45)} = 8.45$, $p < 0.0001$). At Foundiougne a similar pattern emerged, *RBF* was significantly higher in October than in April (ANOVA, $F_{(8, 111)} = 2.04$, $p < 0.05$) (Figure 5.5**;** Appendix Table A).

5.3.5. Reproductive parameters and the environment

The applied multiple linear regression models revealed concerted effects of temperature and salinity explaining 90%, 25%, and 97% of the observed variances in *RBF*, K_{hyd}, and I_G, respectively. Temperature and salinity had opposing effects on *RBF*. While temperature had a positive and quadratic effect on the square root transformed data on *RBF*, the relationship between salinity was negative and quadratic (Table 5.2). The apex point of the function was determined for waters warmer than 30°C and at salinities of around 46. The model results on I_G also revealed mirroring effects of temperature and salinity on the ratio of gonad weight to female body weight. In both models the intercept did not pose as a significant term. Conversely, temperature had a linear and negative effect on K_{hyd} in *E. fimbriata*, while salinity exhibited a negative and quadratic effect on this somatic energy storage parameter. A minimum in K_{hyd} became apparent in females spawning in waters with surface temperatures around 30°C at a salinity of 46 (Figure 5.6).

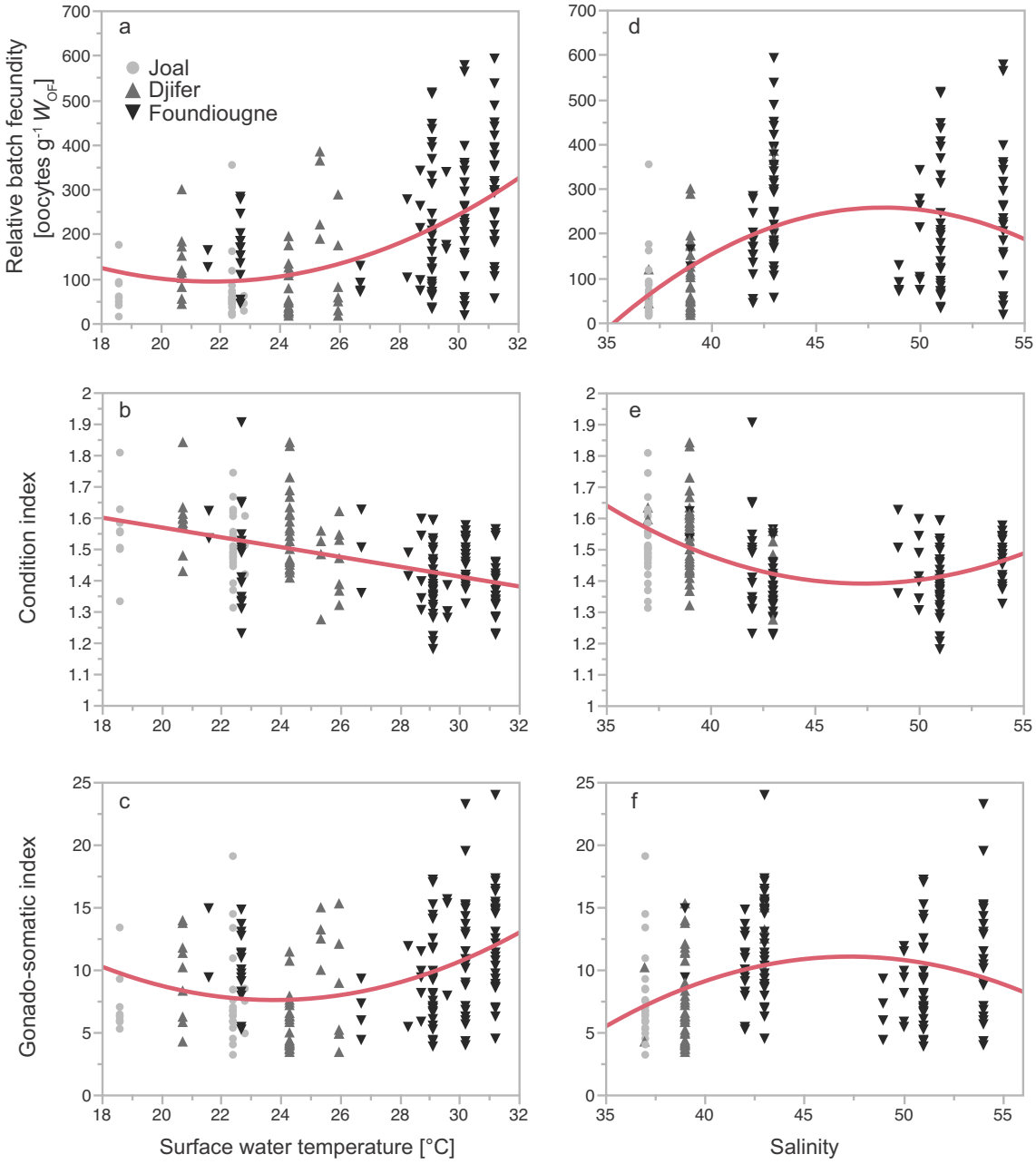

Figure 5.6 Relationships between relative batch fecundity, condition index, and gonado-somatic index with surface water temperature (a, b, c) and surface water salinity (d, e, f) in *Ethmalosa fimbriata*. Females were sampled at Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches). W_{OF}: Ovary free body weight

Table 5.2 Results of the multiple linear regression models of relative batch fecundity, condition index, and gonado-somatic index in *Ethmalosa fimbriata* against temperature (T, T^2), salinity (S, S^2). Estimates of the parameters for the least adequate models, including only significant terms, are shown, together with their respective standard errors and p-values. RSE: Residual standard error, SE: Standard error

	Relative batch fecundity			**Condition index**			**Gonado-somatic index**		
	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p
Intercept				2.1680917	0.3079701	<0.0001			
T	-5.725703	1.254891	<0.0001	-0.0053596	0.0014593	<0.001	-0.5523669	0.1249885	<0.0001
S	3.378869	0.718607	<0.0001	-0.0365113	0.0143465	<0.05	0.3900672	0.0715741	<0.0001
T^2	0.118154	0.023322	<0.0001				0.0108684	0.0023229	<0.0001
S^2	-0.035223	0.007655	<0.0001	0.0004007	0.0001541	<0.05	-0.0041510	0.0007624	<0.0001
R^2		0.9023			0.2544			0.9646	
RSE		4.1560			0.0458			0.4140	

5.4. Discussion

Understanding the spawning biology of fish species in extreme environments, such as inverse hypersaline estuaries, is crucial for evaluating the reproductive potential of stocks at the outer ranges of their physiological capacity (Morgan 2008, McBride et al. 2015). For the first time in marine fishes, the optimal salinity for egg production was observed to be in hypersaline waters (salinity 42 - 51). Obtained results on fecundity variations in *Ethmalosa fimbriata* suggest that the species is well adapted to extreme fluctuations in environmental conditions through highly variable reproductive output. However, the observed increase in reproductive effort under hypersaline conditions is accompanied by a trade-off between batch fecundity, gonadal energy storage, and somatic growth.

The length spectrum of females sampled for the present study is comparable to the ones obtained in former studies conducted in the same geographical area (Charles-Dominique 1982, Panfili et al. 2004). The observed differences in length/weight relationships revealed that females sampled in the Saloum River's middle reaches (Foundiougne) generally had a lower weight at a certain length when compared to their counterparts sampled at the Saloum River's mouth (Djifer) and at the Senegalese South Coast (Joal). Females (stage I - IV) sampled at Foundiougne were in significantly worse nutritional condition (*K*) in all months when compared to specimens sampled at Joal and Djifer. In specimens sampled inside the estuary seasonal differences in *K* became apparent with notable peaks in February (cool and dry season) and July (warm and dry season). This may hint towards a shift from a marine to a hypersaline phytoplankton community, and thus to temporal differences in food item quality/abundance (Nche-Fambo et al. 2015, CHAPTER II). Also, females spawning in the estuary's middle reaches were exhibiting lower condition indices (K_{hyd}) (1.37 ± 0.08) than the ones reproducing in the open ocean (1.56 ± 0.12). It has been proposed before that mature fish might travel upstream to spawn, whereas juveniles and immature females may instead tend to move downstream to forage under marine conditions (Blay Jr. & Eyeson 1982b, Charles-Dominique 1982, Diankha et al. 2013). An energetically expensive migratory behaviour towards spawning grounds has been described for the anadromous American shad *Alosa sapidissima* (Wilson, 1811) (Leggett & Carscadden 1978, Leggett & Trump 1978, Glebe & Leggett 1981), and for the Pontic shad Alosa immaculata (Bennett, 1835) (Navodaru & Waldman 2003). The expected travelling distances of female *E. fimbriata* in West African waters, however, are much smaller than those reported for Alosa species in North America or Eastern Europe and are therefore not necessarily suitable to explain this discrepancy in somatic energy budget. High energetic costs for osmoregulation processes under hypersaline conditions within the middle reaches of the Saloum River and/or differences in stock densities between the sea and the estuary might rather serve as explanations for the observed differences in condition index (Panfili et al. 2004, Casini et al. 2011). Still,

elevated stock sizes appear to have negative effects on fecundity in clupeids (Haslob et al. 2011).

Spawning females were observed at Foundiougne during the entire study period, at Djifer from February until July, and at Joal from March until May. These results are in good agreement with the species' temporally limited spawning periods defined in past studies (Scheffers et al. 1972, Panfili, Durand, et al. 2004). It has been suggested before that seasonally varying hydrographic conditions (changes in water temperature and salinity) play a critical role in the timing and intensity of spawning in clupeid fishes (Isaac-Nahum et al. 1988, Tsikliras & Antonopoulou 2006, Coombs et al. 2006). Locally varying spawning times therefore hint towards favourable hydrographic condition for offspring survival at the coast after the onset of upwelling at the beginning of the year. Given the species' prolonged spawning season, however, conditions are most likely favourable for spawning and early life stages survival in the Saloum River's middle reaches all year around. Thus, an examination of *E. fimbriata*'s batch fecundity in relation to respective physical parameters could shed some light on observed energetic disparities and the species' spawning tactic.

High variances in absolute batch fecundity (*ABF*) were previously reported for *E. fimbriata* throughout the entire West African coast (Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982a, Kusemiju & Onadeko 1990). Our results further underline the species' high phenotypic plasticity in this particular reproductive trait (Jennings & Beverton 1991, Ghalambor et al. 2007). Further, females sampled at Foundiougne produced significantly more hydrated oocytes at a certain length/weight than their counterparts sampled at Djifer and Joal. Thus, females spawning inside the Saloum River's middle reaches were more fecund for the same size. A closer look at the observed spatial and monthly variability in relative batch fecundity (*RBF*) might explain the observed differences in *ABF*. Comparable to past studies, calculated values for mean RBF also varied widely between months (Albaret & Gerlotto 1976, Charles-Dominique 1982, Panfili et al. 2004). Females sampled at elevated salinities were producing four times more oocytes (294 ±137 oocytes g^{-1} ovary free body weight [W_{OF}]) than their oceanic counterparts (74 ± 65 oocytes g^{-1} W_{OF}). Clupeid fishes are known to adjust reproductive tactics to local conditions (Leggett & Carscadden 1978, Irwin & Bettoli 1995, Blaber et al. 1996), and thus produce egg quantities and egg sizes that favour offspring survival under the environmental conditions in which they are to develop (Kamler 2005, Leal et al. 2009).

Length-specific fecundities of experimentally starved Pacific herring *Clupea pallasii pallasii* (Valenciennes, 1847) were similar to those of fed fish, but the unfed fish had higher weight-specific fecundities, corresponding to a greater loss of somatic tissue during captivity (Hay & Brett 1988). Consequently, calculated *RBF* was highest among the unfed/starving fish. In the case of *E. fimbriata* elevated *RBF* as adaptive response to starvation is unlikely, since no

contradictory effect of female size on *ABF* could be observed. Additionally, primary production within tropical mangrove ecosystems has been amply described as comparably high and thus a shortage in food items is unlikely (Pagès & Gadel 1990, Jennerjahn & Ittekkot 2002).

For estimating the correlation of spawning parameters and water temperature we used satellite derived sea surface temperatures as well as in situ surface temperatures for inland waters. Due to the range in observed surface water temperatures from 18 - 31°C we accepted the satellite derived values with an accuracy of ± 0.5°C for our purposes (Castillo & Lima 2010). The multiple linear regression models for *RBF* and gonado-somatic index (I_G) indicate that *E. fimbriata* is producing increasing numbers of oocytes, and in consequence the ratio of gonad weight to ovary free body weight is increasing along a rising temperature and salinity gradient. The negative, quadratic effects of temperature and salinity on *RBF* and I_G on the other hand illustrate that elevated metabolic rates and osmoregulation processes play a crucial role in limiting the species reproductive potential under hypersaline conditions. Because K_{hyd} is negatively correlated with temperature and salinity a trade-off effect between batch fecundity/gonadal energy storage and female's somatic condition can be concluded. Further evidence is provided by the fact that mean monthly condition of stage V females decreased significantly while mean *RBF* increased in concert with rising temperature and salinity gradients throughout most of the sampling period. At Foundiougne spawner condition is only improving again in October when salinity is decreasing due to the prevailing rainy season. These observations lead to the conclusion that the reproductive investment of well-adapted clupeids is not necessarily dependant on fish size, but rather on the respective spawning environment. A high variability in reproductive output through phenotypic plasticity seems to allow fast adjustment to drastically varying environments, and may therefore be beneficial for recruitment in clupeids (Leggett & Carscadden 1978, Haslob et al. 2011).

Obtained results give strong evidence that elevated salinities (42 - 51) in synergy with high water temperatures (26 - 30°C) directly affect condition, the reproductive investment, and consequently the reproductive potential of mature female *E. fimbriata*. For the first time in clupeid fishes, the current study shows a spawning optimum at salinities that far exceeded marine conditions. Conversely, the marine Brazilian menhaden *Brevoortia aurea* (Spix & Agassiz, 1829) population exhibits a spawning preference at salinities which are far lower than marine (salinity 10 - 25) in order to ensure the retention of early life stages at the estuary's mouth where offspring survival chances are most likely higher (Acha & Macchi 1999).

Embryonic developmental times and hatching rates are not only affected by water temperatures, but also by salinities (Lambert et al. 2004). While former studies showed that survival of early life history stages is possible in either high

temperature (Pepin 1991) or high salinity regimes (Holliday 1969), egg development, hatching, and larval survival are prone to impairment under combined high temperature and high salinity conditions (Hempel 1979, Martin 1988). Certain temperature and salinity combinations might trigger reproduction in clupeids inhabiting variable hypersaline environments, comparable to the isolated effect of temperature on temperate (Coombs et al. 2006), or even on other tropical species (Milton et al. 1995). The observed reproductive tactic may therefore aim towards spawning in a favourable environmental window (Cury & Roy 1989, Motos 1996), ensuring survival of well-adapted offspring through enhanced quality in food items and/or predator avoidance during the consecutive weeks (May 1974). On the contrary, it may also be hypothesised that this tactic might overcompensate for high offspring mortality under these rather extreme conditions (Rickman et al. 2000).

In consequence, spawning effort combined with elevated metabolic rates under high temperature and energy allocation to osmoregulation processes under hypersaline conditions are most likely too high to be compensated by nutritional intake and are therefore at the expense of somatic growth in clupeids (Leonard et al. 1999, Sangiao-Alvarellos et al. 2003). This trade-off mechanism will ultimately limit the reproductive potential of clupeid fishes inhabiting estuarine environments impacted by global change. The observed high variability in reproductive potential of *E. fimbriata* seems to be a viable tactic in order to adjust to the drastically changing and rather extreme environments such as hypersaline estuaries. Future examinations of food webs in hypersaline environments in combination with laboratory experiments on egg survival and developmental rates under different temperature and salinity regimes are necessary to understand underlying mechanisms. Even further, future application of a Dynamic Energy Budget model may foster our understanding of the observed discrepancies. It would allow for a closer investigation of energy allocations to determine to which degree they are associated with water temperature and salinity in this species (Pecquerie et al. 2009).

5.5. Conclusion

Even though hydrographic conditions in the middle reaches of the Saloum River are favourable for spawning in *Ethmalosa fimbriata* all year around, temporal and spatial variations in spawner condition, gonado-somatic index, and batch fecundity could be observed and linked to heterogeneous environmental conditions. Our results show that *E. fimbriata* has to budget a significant amount of energy towards elevated metabolic rates and osmoregulation processes in an effort to spawn under high temperature/hypersaline conditions. The observed trade-off mechanism between somatic energy budget and reproductive investment ultimately limits the species' reproductive potential inside the estuary. Due to its high adaptive potential and euryhaline physiology *E. fimbriata* is so far likely to benefit from the severe impacts of climate change on its spawning habitat by potentially

outcompeting other pelagic fish species. However, a further decrease in precipitation and a concerted elevation of ambient salinities will have negative impacts on the species' habitat size, reproductive potential, and eventually on its stock size.

5.6. Acknowledgments

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CHAPTER II

6. USING OOCYTE ESSENTIAL FATTY ACID COMPOSITION TO ASSESS SPAWNER REPRODUCTIVE POTENTIAL UNDER HYPERSALINE CONDITIONS

Julian Döring and Werner Ekau

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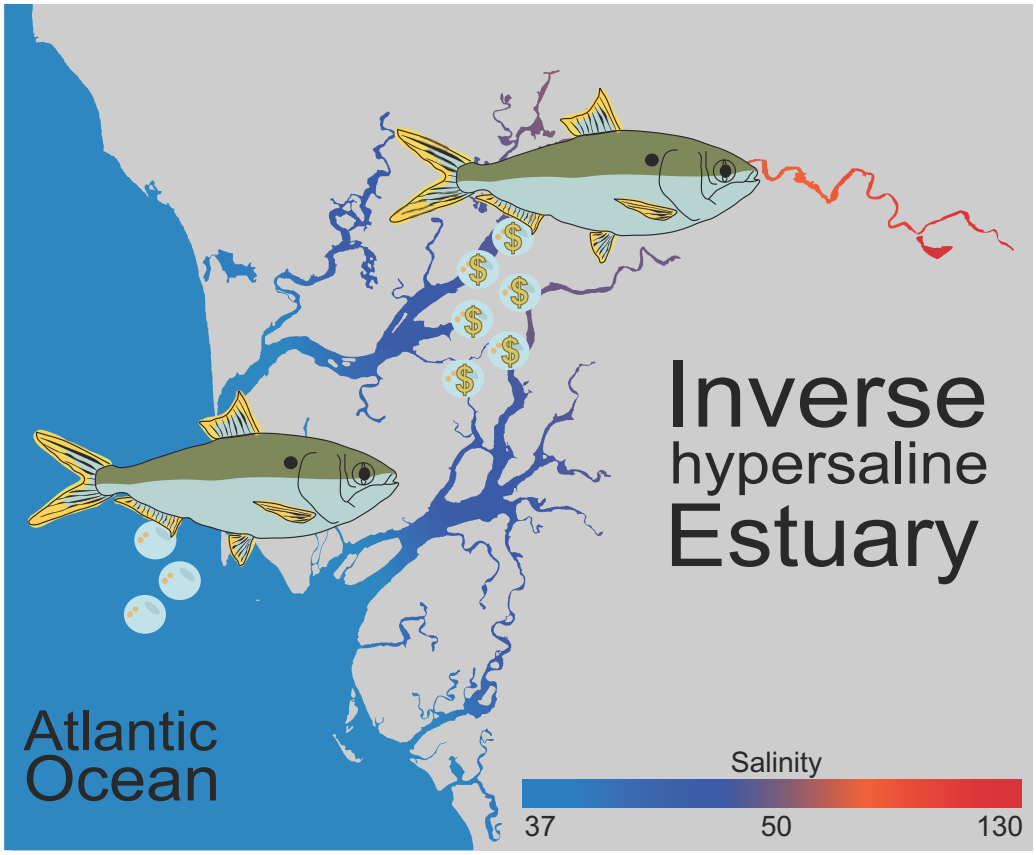
Inverse
hypersaline
Estuary
Atlantic
Ocean
Salinity
37
50
130

Abstract

Inversion of estuaries in the dry tropics (i.e. salinity increases upstream) against the backdrop of a globally changing climate is forcing migratory clupeid fish species to adapt quickly to modifications in their spawning habitats to ensure reproductive success. Hatching success and survival probabilities of marine fish eggs and early life stages are prone to impairment under high temperature and elevated salinity conditions. Essential fatty acids affect osmoregulation in marine fish early life stages, and deficiencies can therefore be expected to cause elevated offspring mortality under hyperosmotic conditions, ultimately modifying a stock's reproductive potential. Here we show that oocytes spawned by bonga shad *Ethmalosa fimbriata* inside a hypersaline estuary exhibited significantly higher 20:4 (n-6) proportions (1.6 ± 0.7% of total fatty acids) than oocytes spawned under marine conditions (0.6 ± 0.2%). Further, oocyte proportions in 20:4 (n-6), 20:5 (n-3), and 22:6 (n-3) were positively correlated with water temperature, water salinity, and female reproductive investment (relative batch fecundity, gonado-somatic index, oocyte dry weight). Oocytes spawned during high temperature/high salinity conditions inside the estuary are therefore likely to develop normally under the prevailing conditions. Reproductive potential was determined to be highest in females spawning inside the estuary at the end of the wet season, when temperatures are high and salinities are less stressful due to freshwater input. Results lead to the conclusion that migratory clupeid fishes can maintain their reproductive potential in hypersaline estuaries and lagoons, which potentially allows for sustainable stable stock sizes.

Keywords: Arachidonic acid · Clupeidae · Fatty acids · Inverse estuary · Maternal effects · Reproductive investment · Salinity · Temperature

6.1. Introduction

The bonga shad *Ethmalosa fimbriata* (Bowdich, 1825) is a clupeid fish species inhabiting West African coastal and estuarine waters from Mauritania to Angola. It is one of the most important fishes targeted by artisanal fisheries in Cameroon, Ivory Coast, Nigeria, and Senegal (Charles-Dominique & Albaret 2003). Spawning of *E. fimbriata* in Senegalese coastal waters mainly takes place during the upwelling season from February to May (Charles-Dominique 1982). In the Sine Saloum estuary, the batch-spawning species exhibits a protracted reproductive period that usually lasts from January to August (Albaret & Gerlotto 1976, Panfili et al. 2004). Its physiology, which is adapted to eurysaline conditions allows the species to cope with varying salinities from around 0 to 97 (Panfili et al. 2006) and even to reproduce in waters with salinities up to 66 (Charles-Dominique & Albaret 2003). *E. fimbriata* is considered a passive filter feeder and undergoes an ontogenetic diet shift, with copepods representing the main prey item for larvae and phytoplankton for adults (Facade & Olaniyan 1972, Blay Jr. & Eyeson 1982b, Lazzaro 1987). Past studies indicate that the species additionally feeds on benthic deposits such as organic detritus and diatoms (Bainbridge 1963, Facade & Olaniyan 1972).

The Sine Saloum estuary is an inverse hypersaline environment with seasonally changing salinities (Pagès & Citeau 1990). In the estuary's middle reaches, water temperatures regularly exceed 30°C and salinity surpasses 50 by the end of the dry season in June/July (Simier et al. 2004). *E. fimbriata* exhibits significantly higher batch fecundity when spawning in these hypersaline conditions compared to marine water or freshwater (Scheffers et al. 1972, Albaret & Gerlotto 1976, Panfili et al. 2004).

The eggs of *E. fimbriata* are pelagic and after fertilization spend 3 to 9 h in the water column until hatching (Albaret & Gerlotto 1976). Incubation times, however, may vary significantly with water temperature (Jatteau et al. 2017) and salinity (Holliday 1969). Hatching success and survival probabilities of marine fish eggs and early life stages are generally prone to impairment under high temperature and elevated salinity conditions (Hempel 1979, Martin 1988). Thus, maternal effects become increasingly important to ensure offspring survival under extreme environmental conditions. In this regard, the nutritional quality of oocytes, especially in terms of their fatty acid (FA) composition, becomes an important factor in the ontogeny and embryonic development of clupeid fishes in stressful environments (Tocher & Sargent 1984, March 1993, Tocher 2010). Because of a globally changing climate and the possible inversion of estuaries in the dry tropics (Wolanski 1986, Pagès & Citeau 1990, Ridd & Stieglitz 2002, Wedderburn et al. 2016), migratory clupeid fish species have to be able to adapt quickly to rapid changes in their spawning habitats to ensure reproductive success.

FAs are reported to have a strong impact on egg quality, ultimately modifying offspring viability (Castro et al. 2010, Patterson & Green 2015). FAs such as arachidonic acid (20:4 [n-6]; ARA), eicosapentaenoic acid (20:5 [n-3]; EPA), and docosahexaenoic acid (22:6 [n-3]; DHA) cannot be synthesized de novo by marine fishes in significant quantities and for this reason are considered essential fatty acids (EFA) (Sargent et al. 1995). Marine primary producers and some heterotrophic flagellates possess enzymes to desaturate (n-3) and (n-6) polyunsaturated fatty acids (PUFA) through shorter precursors such as oleic acid (18:1 [n-9]) and linoleic acid (18:2 [n-6]) (Dalsgaard et al. 2003). Thus, fishes need to acquire EFA either directly, via consumption of phytoplankton, or indirectly, via consumption of organisms which fed on phytoplankton (e.g. zooplankton) (Ackman & Jangaard 1964, Lee et al. 1971, Fuiman et al. 2015).

Increasing water temperatures will elevate levels of ARA and saturated fatty acids (SAFA) but lower EPA and DHA contents in marine phytoplankton. This is probably caused by a decrease in enzymatic activity due to lower oxygen availability and the need to maintain cell membrane stability at higher temperatures (Hixson & Arts 2016). Also, phytoplankton community composition changes under hypersaline conditions, as found in inverse estuaries (Nche-Fambo et al. 2015). Therefore, water temperature and salinity are suspected to have direct effects on the FA composition in food items relevant for *E. fimbriata* (Bainbridge 1963, Blay Jr. & Eyeson 1982b). In clupeoid fishes, the EFA produced by phytoplankton are incorporated into the intermediate tissues, gonads, and eventually oocytes during the spawning season (Linko et al. 1985, Shirai et al. 2002, Garrido et al. 2007, Castro et al. 2010). Elevated levels of ARA, DHA, EPA, and the DHA/EPA ratio in oocytes accounted for an increased hatching success in a variety of fish species (Leray et al. 1985, Pickova & Dutta 1997, Lane & Kohler 2006, Patterson & Green 2014, Asil et al. 2017). Additionally, EFA play an important role in larval osmoregulation (Bransden et al. 2004). ARA and EPA are substrates for the synthesis of hormone-like compounds called eicosanoids (Bell et al. 1994, Tocher 2003), which are involved in reproduction, hormone release, cardiovascular function, neural function, and osmoregulation in a variety of organisms (Mustafa & Srivastava 1989). In synergy with hormones, the subfamily prostaglandin modulates the ion and electrolyte balance in the kidney and gills of fishes (Horseman & Meier 1978, Brown & Bucknall 1986, Beckman & Mustafa 1992). Enhanced dietary intake of ARA thus increases prostaglandin production (Bransden et al. 2004) and thereby the resistance to hyperosmotic stress in a variety of fish larvae (Koven et al. 2003, Willey et al. 2003). Also, the ratio of (n-3) to (n-6) PUFA in the gill lipids of European eels *Anguilla anguilla* (Linnaeus, 1758) reared in seawater was three-fold higher than in individuals reared in freshwater, enhancing their ability to maintain osmotic equilibrium in waters of different salinities at different temperatures (Thomson et al. 1977, March 1993). All these findings imply that a change in the FA composition of phytoplankton communities might severely affect the reproductive potential and ultimately the recruitment success of clupeid fish species reproducing in hypersaline environments.

For the first time, we used EFA to assess the reproductive potential of a clupeid fish, *E. fimbriata*, under increased salinity conditions. We hypothesized that oocyte quality in terms of FA composition is related to the individual's reproductive investment (batch fecundity, gonado-somatic index (I_G), gonad weight, oocyte dry weight (*ODW*), oocyte volume (*OV*)) and influenced by ambient temperature and salinity conditions. We chose *E. fimbriata* from the Sine Saloum in Senegal, as this estuary exhibits a wide range of elevated salinities in which reproduction of the species had been observed.

6.2. Materials & Methods

6.2.1. Study area

Located 100 km south of Dakar (between 13°55' and 14°10' N, and 16°03' and 16°50' W), the Sine Saloum estuary is comprised of 3 main branches, the Saloum, the Diomboss, and the Bandiala. An extended dry season, with cool temperatures from November to March and warm temperatures from April to June, is characteristic for this region. July to October is considered the wet and warm season (Simier et al. 2004). Total rainfall has been decreasing since the 1920s, with a particularly severe decline since 1961. Combined effects of tides, reduced river run-off, intense evaporation, and shallow waters in the estuary's mouth have led to high salinity and overall inversion of the salinity gradient (Pagès & Citeau 1990).

Monthly sampling was conducted at the coast and inside the estuary from February to October 2014, during *Ethmalosa fimbriata*'s main spawning period (Charles-Dominique 1982, Panfili et al. 2004). Three different sites were sampled: Joal (coast, 14° 9.1' N, 16° 51.7' W), Djifer (river mouth, 13° 57.8' N, 16° 44.8' W), and Foundiougne (upstream Saloum River, 14° 8.1' N, 16° 28.1' W) (Figure 6.1).

6.2.2. Environmental parameters

The coastal waters off Joal and in the Saloum River are well mixed and do not show significant stratification (Sloterdijk et al. 2017, Tiedemann & Brehmer 2017). Thus, only surface water temperatures and salinities were measured for each sampling week. Water temperatures at Foundiougne were recorded with a digital thermometer. For Joal, where catch positions could only be estimated within a radius of ~15 km, and Djifer, average night-time sea surface temperatures were assessed by means of MODIS satellite data (Aqua, level 2, 0.1°). At Djifer and Foundiougne, salinity was determined with a handheld refractometer using *in situ* water samples. For Joal, monthly means in MODIS satellite-derived (Aquarius, level 3, 0.5°) sea surface salinities were used.

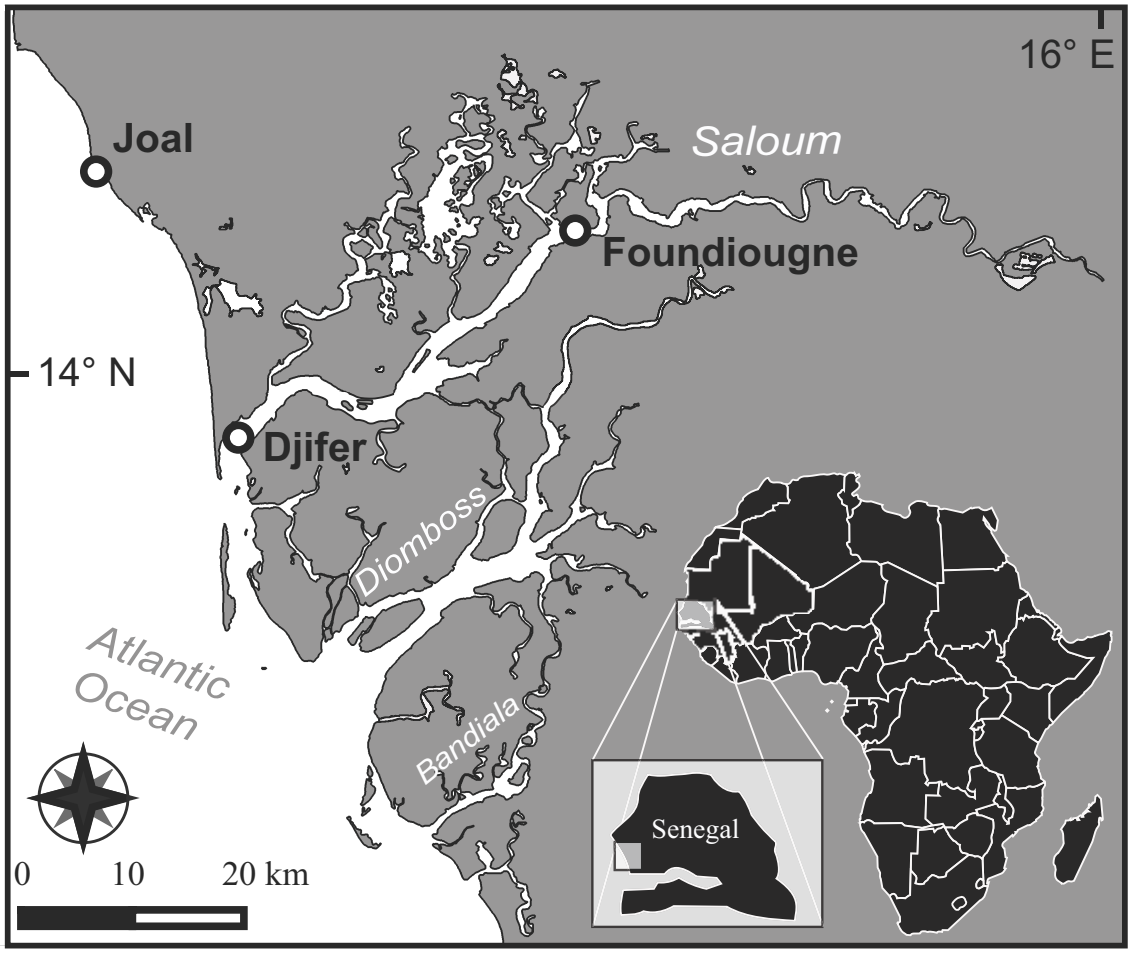

Figure 6.1 Senegalese coast and the Sine Saloum estuary, including sampling sites: Joal (coast), Djifer (Saloum River mouth), and Foundiougne (Saloum middle reaches)

6.2.3. Sample collection

At each sampling site, fish were caught with gill nets (32 - 36 mm mesh size) by local fishermen and immediately stored on crushed ice after landing. Approximately 1000 fish per sampling site and month were examined to find stage V females, i.e. mature individuals with ovaries containing fully hydrated oocytes (Blay Jr. & Eyeson 1982a). Females that had spawned recently or lost part of their egg batch during handling were rejected. Spawners were weighed ($W_W \pm 0.01$ g) and measured (L_T, nearest mm), and their ovaries were dissected carefully. Oocytes were gently extracted from one ovary lobe, rinsed with deionized water, and counted under a stereomicroscope. Around 70 oocytes per fish were counted, transferred to a pre-weighed tin cap, and deep frozen in liquid nitrogen. Dissected ovaries were transferred to a 4% borax-buffered formaldehyde and freshwater solution for fecundity analysis and determination of OV. At Foundiougne, spawning females could be sampled in almost all sampling months, allowing a comparison between dry and wet seasons. No hydrated oocytes for FA analysis were obtained at Joal and

Foundiougne in February. Across all sampling sites and seasons, 96 females were analyzed.

Preliminary data analysis revealed significant spatial differences in oocyte FA composition. It was therefore decided to conduct additional field sampling to obtain basic information on *E. fimbriata*'s feeding habits. This supplementary sampling campaign was conducted in November 2016. Microphytoplankton was collected by casting a handheld net (20 µm mesh size) three times per station. Subsequently, phytoplankton samples were filtered onto micropore filters (Whatman) and transferred to cryovials. The stomach contents of 10 female fishes per sampling site and the counted oocytes of 12 females were transferred into pre-weighed tin caps and also stored in cryovials. All samples were immediately conserved in liquid nitrogen and stored at - 80°C in the laboratory.

6.2.4. Spawner reproductive investment

Oocytes in tin caps were freeze dried (24 h) and weighed again to ascertain their dry weights (*ODW* ± 0.1 µg). Ten oocytes per ovary fixed in 4% formalin were photographed under a stereomicroscope and measured along their minor (d_1) and major (d_2) diameters using the calibrated measurement tool in Image J 1.8.0 (http://rsb.info.nih.gov/ij/). Following Alderdice et al. (1979), subsequent equation was employed to calculate oocyte volumes (*OV*, mm^3) (Equation 6.1):

$$OV = \frac{4}{3}\pi\left(\frac{d_1}{2}\right)^2\left(\frac{d_2}{2}\right) \quad (6.1)$$

The gonado-somatic index (I_G) of female spawners was calculated by dividing the ovary weight (W_O ± 0.0001 g) by the ovary-free body weight (W_{OF} ± 0.1 g) (Zydlewski et al. 2003) as follows (Equation 6.2):

$$I_G = \frac{W_O}{W_{OF}} \times 100 \quad (6.2)$$

Absolute batch fecundity (*ABF*) was estimated gravimetrically using the hydrated oocyte method for indeterminate spawners (Hunter et al. 1985). Relative batch fecundity (*RBF*) of the females was calculated by dividing *ABF* by W_{OF} (Alheit 1988).

6.2.5. Fatty acid analyses

Lipid extraction was performed using a 2:1 solvent mix of dichloromethane/methanol. The deuterated FA 12:0 (Larodan, 20 ng μl^{-1}) was added as an internal standard and a deuterated 22:0 FA standard (Larodan, 20 ng μl^{-1}) as an esterification efficiency control. Samples were homogenized using ultrasound. Esterification was performed for 8 h at 80°C in 250 µl hexane and 1 ml 3% sulphuric acid/methanol solution. All solvents used were gas chromatography (GC) grade. The fatty acid methyl esters (FAME) were analysed in 1 ml aliquots injected in an Agilent Technologies 7890B GC system equipped with a polar column (Macherey-Nagel, OPTIMA 225, 30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (base temperature 260°C) with helium as a carrier gas at a constant flow of 1 ml min^{-1}. The column oven was initially set to 40°C and then heated at a 100°C min^{-1} increase to 112°C for 2 min, at 14°C min^{-1} to 125°C, at 5°C min^{-1} to 160°C, at 20°C min^{-1} to 180°C, at 3°C min^{-1} to 200°C, at 4°C min^{-1} to 240°C for 15 min, and at 20°C min^{-1} to 260°C, which was held for 5 min. The system was calibrated with a 20 component marine oil FAME mix (Restek), and chromatograms were analysed using MassHunter Work Station - Quantitative Analysis (Agilent, Version B.07.00) GC software. Three random samples were processed via GC mass spectrometry (Macherey Nagel, OPTIMA FFAplus, 30 m × 0.25 mm × 0.25 µm, with identical oven parameters as above) to verify FA profiles. To ensure comparability with past studies, results for FA are given as a percentage of the combined weights of all detected FA (Castro et al. 2010).

6.2.6. Statistical analyses

Analysis of variance (ANOVA) was carried out with FA (as % of total FA), *ODW*, *OV*, and *RBF* as dependent variables and sampling site, sampling month, and sampling season as independent variables. Given that ANOVA techniques are quite robust against small sample sizes (Brown & Forsythe 1974) and the violation of the normality assumption (Schmider et al. 2010), data were statistically tested by ANOVA if at least the assumption of homogeneity of variances was met. Data that did not meet the assumption of homogenous variances (Levene's test) were square root transformed. In cases where significant differences were detected, post hoc multiple comparisons were performed (Tukey's HSD).

Multiple linear regression models were applied to assess the responses of *RBF* and *ODW* to temperature (T, T^2), salinity (S, S^2), and the interaction between both variables ($T \times S$). Additionally, the responses of the FAs 18:1 (n-9), ARA, EPA, and DHA to the physical parameters, as well as to either *RBF*, I_G, or W_{OF} and W_O, were investigated. Non-significant lower-order terms were kept in the model. The resulting minimal adequate models (as determined by corrected Akaike's information criterion) were tested for the constancy of variances (residuals against fitted values) and normal distribution of their residuals (Shapiro-Wilk test). Where any of these two assumptions were violated, the response variables were log transformed. This was the case for *RBF* and for the EFA ARA, EPA, and DHA. To illustrate spatial and seasonal differences in

oocyte FA profiles, a principal component analysis (PCA) was performed. Relative values for the most abundant FA and EFA were log transformed. All statistical analyses were carried out using JMP 10.0.1 (SAS Institute, www.jmp.com).

6.3. Results

6.3.1. Environmental conditions

Night-time surface water temperatures at the three sampling sites steadily increased throughout the sampling period. Surface temperatures were higher at Foundiougne than at Joal in all sampled months in 2014, with intermediate values recorded at Djifer. However, surface water temperature was highest at Joal (27.8°C) when compared to Djifer (27.2°C) and Foundiougne (26.8°C) in November 2016. At the beginning of the sampling period (February 2014), salinity was determined to be 39 at Foundiougne as well as at Djifer. In the following months, salinity increased steeply at Foundiougne, eventually peaking in July at 54 (end of the dry season) until it dropped to 51 in August (beginning of the wet season) and subsequently to 43 in September and October (end of the wet season). At Djifer, salinity peaked at 43 in July and August before dropping to 32 in September. Coastal salinity at Joal fluctuated around 37 throughout the entire sampling period. Salinity at Joal, Djifer, and Foundiougne in November 2016 was determined to be 37, 34, and 40, respectively (Figure 6.2).

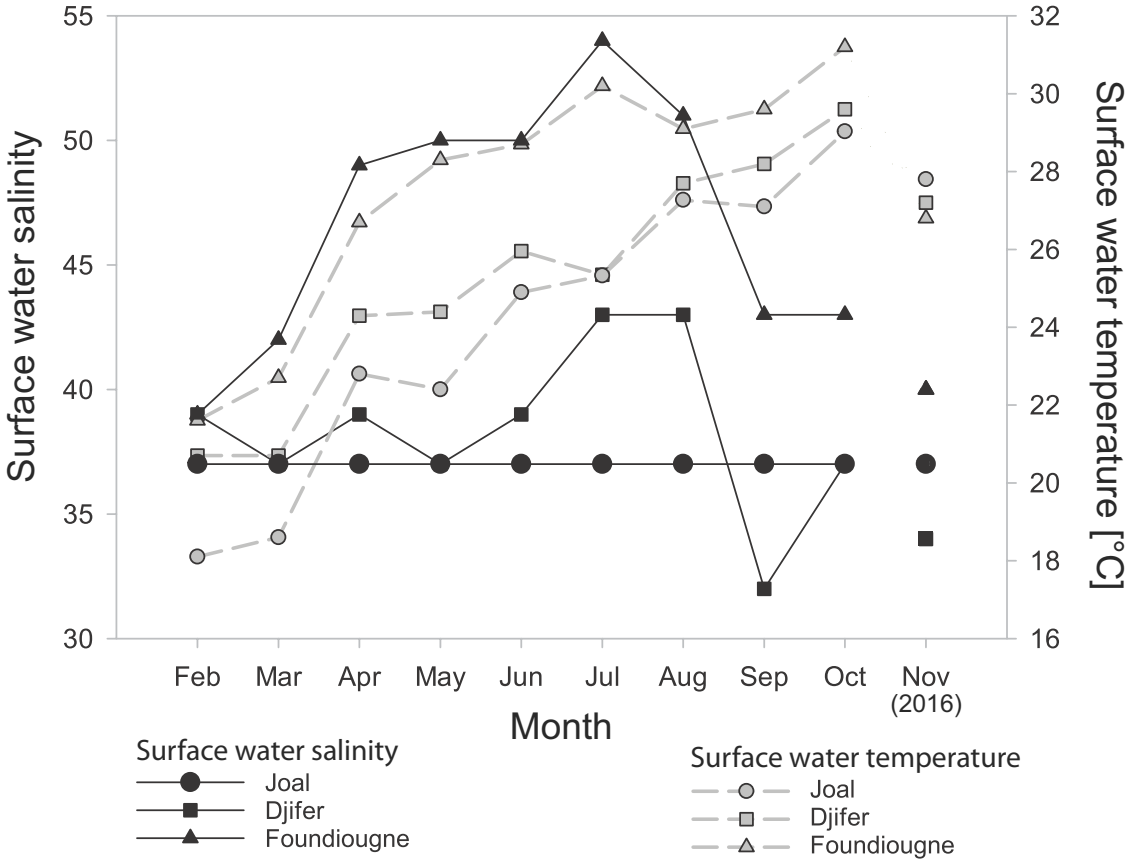

Figure 6.2 Surface water temperatures and surface water salinities as recorded at Joal, Djifer, and Foundiougne in 2014 and November 2016

6.3.2. Spawner reproductive investment

RBF in female *Ethmalosa fimbriata* sampled within the Saloum River (Foundiougne, Djifer) was significantly higher than in specimens sampled at the coast (Joal; ANOVA, $F_{(3, 68)} = 9.4305$, $p < 0.0001$). I_G of fecund females was significantly higher at Foundiougne during the wet season when compared to their counterparts sampled at Joal (ANOVA, $F_{(3, 70)} = 5.4748$, $p < 0.01$). *ODW* at Joal and Djifer did not vary substantially between sampling months. The dry weight of oocytes sampled at Foundiougne, however, peaked in April and decreased significantly during the progression of the sampling period, reaching a minimum in October (ANOVA, $F_{(7, 40)} = 22.5591$, $p < 0.0001$; Figure 6.3). Hence, *ODW* was significantly higher in oocytes sampled at Foundiougne during the dry season when compared to the other sites and seasons (ANOVA, $F_{(3, 67)} = 7.7573$, $p < 0.001$). While no monthly differences in *OV* were detected at Djifer and Foundiougne, *OV* at Joal was significantly higher in April than in March (ANOVA, $F_{(2, 11)} = 7.7279$, $p < 0.05$). However, no significant difference in the total weight of all FA could be detected among sites and seasons (Table 6.2).

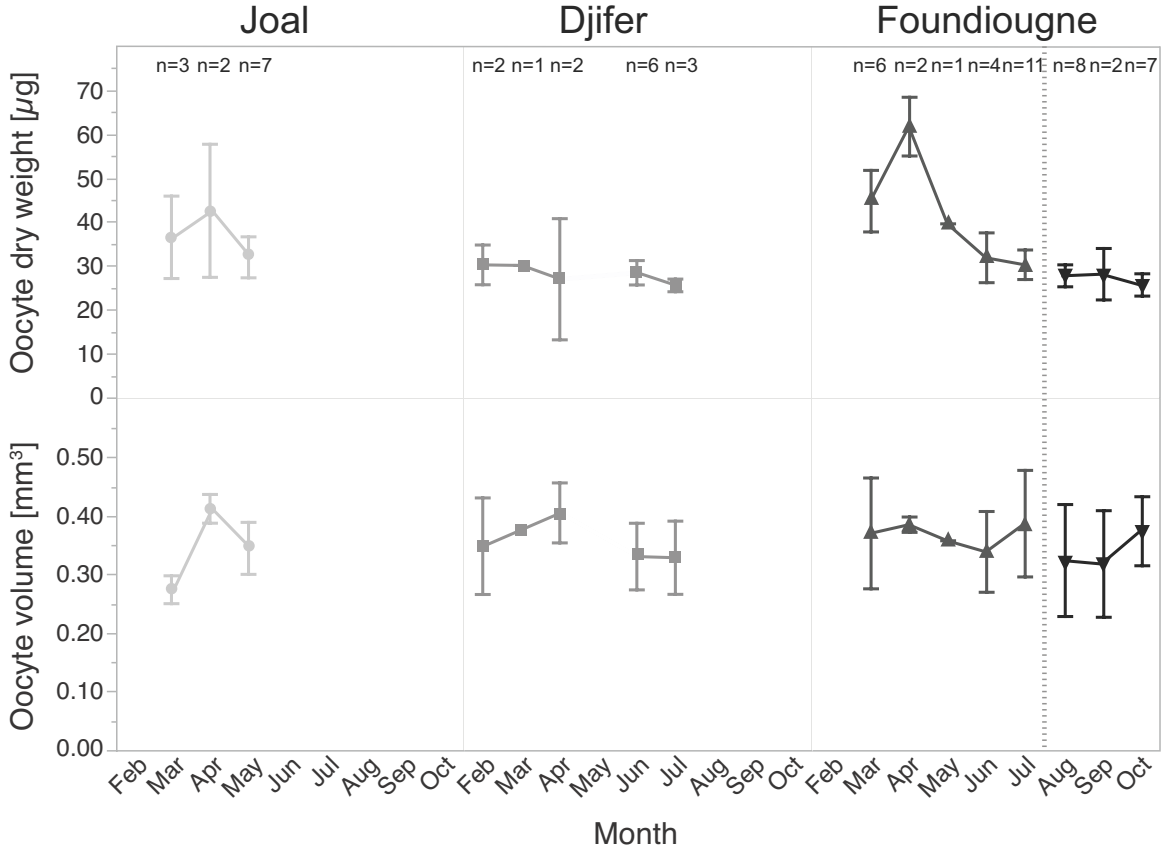

Figure 6.3 Monthly differences in dry weights and volumes (mean ± SD) of *Ethmalosa fimbriata* oocytes at the 3 sampling sites at the Senegalese South Coast (Joal) and inside the Saloum estuary (Djifer, Foundiougne) in 2014

Table 6.1 Mean (± SD) relative batch fecundity (*RBF*) and gonado-somatic index (I_G), oocyte dry weight (*ODW*, µg), oocyte volume (*OV*, mm^3), fatty acids (FA), and their total weight (TFA, µg) in *Ethmalosa fimbriata* oocytes at the three sampling sites (Joal, Djifer, and Foundiougne) and during the two sampling seasons inside the Saloum estuary (dry and wet season). The size ranges of sampled females are given in L_T. FA are expressed as percentage of total FA. For rows in **bold**, significant differences were found by analyses of variances; letters display Tukey's HSD results (values not sharing the same letter are significantly different from each other). SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids: PUFA: polyunsaturated fatty acids; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

	Joal	**Djifer (Dry Season)**	**Foundiougne (Dry Season)**	**Foundiougne (Wet Season)**
Months	Mar - May	Feb - Jul	Mar - Jul	Aug - Oct
n	13	14	27	17
Size range [cm]	23.5 -27.3	20.8 – 26.1	20.9 – 28.3	20.1 – 24.7
RBF	**61 ± 38^{a}**	**165 ± 105^{b}**	**205 ± 140^{bc}**	**283 ± 149^{c}**
GSI	**6.9 ± 2.4^{a}**	**10.3 ± 3.5^{ab}**	**9.7 ± 4.6^{ab}**	**12.4 ± 4.3^{b}**
ODW	**33.1 ± 12.5^{ab}**	**28.8 ± 6.2^{bc}**	**37.1 ±10.8^{a}**	**26.8 ± 2.8^{c}**
OV	0.35 ± 0.06	0.35 ± 0.06	0.37 ± 0.08	0.34 ± 0.08
14:0	4.4 ± 1.6	4.8 ± 1.5	4.1 ± 1.2	4.5 ± 1.6
16:0	49.4 ± 3.9	49.8 ± 8.2	47.1 ± 7.3	45.6 ± 9.6
16:1 (n-7)	6.0 ± 1.7	5.5 ± 2.6	5.8 ± 1.2	7.0 ± 2.4
18:0	12.6 ± 1.1^{a}	11.7 ± 2.1^{ab}	10.9 ± 1.7^{b}	12.5 ± 3.0^{ab}
18:1 (n-9)	12.3 ± 4.2^{a}	9.3 ± 3.5^{ab}	10.8 ± 2.2^{a}	8.4 ± 2.0^{b}
18:1 (n-7)	4.2 ± 0.8	4.0 ± 1.6	3.4 ± 0.7	3.9 ± 0.7
18:2 (n-6)t	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.3	0.7 ± 0.3
20:1 (n-9)	0.7 ± 0.6	1.4 ± 2.4	1.2 ± 1.8	1.1 ± 2.1
20:2 (n-6)	1.0 ± 1.4	0.7 ± 0.8	0.4 ± 0.4	0.4 ± 0.4
20:4 (n-6) (ARA)	**0.6 ± 0.2^{a}**	**0.8 ± 0.4^{ab}**	**1.1 ± 0.6^{b}**	**1.6 ± 0.7^{c}**
20:5 (n-3) (EPA)	2.2 ± 0.6	3.2 ± 2.2	3.5 ± 2.3	3.9 ± 2.3
22:1 (n-9)	0.2 ± 0.4	0.4 ± 1.1	0.7 ± 0.9	0.6 ± 1.2
22:6 (n-3) (DHA)	3.9 ± 2.6	6.1 ± 4.7	7.8 ± 5.8	7.4 ± 5.3
24:0	0.5 ± 0.5	0.5 ± 0.3	0.6 ± 0.3	0.9 ± 1.0
24:1 (n-9)	0.7 ± 0.3	0.6 ± 0.4	1.0 ± 2.0	0.7 ± 0.6
Σ (n-3) / Σ (n-6)	3.0 ± 1.3	5.2 ± 5.4	5.0 ± 2.2	4.0 ± 1.6
22:6 (n-3) / 20:5 (n-3)	1.6 ± 0.5	1.8 ± 0.9	2.2 ± 0.6	1.8 ± 0.5
Σ SAFA	67.2 ± 5.8	67.1 ± 10.5	63.1 ± 9.2	63.8 ± 11.7
Σ MUFA	24.1 ± 3.1	21.2 ± 5.0	23.1 ± 2.1	21.7 ± 4.6
Σ PUFA	8.6 ± 4.4	11.7 ± 7.1	13.7 ± 8.3	14.13± 8.5
Σ Other FA	0.6 ± 02	0.6 ± 0.3	0.8 ± 0.5	0.7 ± 0.8
TFA	1.3 ± 0.8	0.8 ± 0.4	1.5 ± 1.2	0.8 ± 0.6

6.3.3. Oocyte fatty acid compositions

SAFA made up the largest portion of the FA at all three sampling sites, followed by monounsaturated fatty acids (MUFA) and PUFA. SAFA and MUFA proportions did not vary substantially among sampling sites and seasons. The proportions of PUFA were higher at Foundiougne (during the dry and wet season) than at Joal and Djifer, albeit not significantly. The SAFA 16:0 (palmitic acid) constituted the largest mean percentage of the oocytes, followed by either 18:0 (stearic acid) or 18:1 (n-9) (oleic acid). The three most abundant FA constituted between 66 and 71% of the total. Proportions of 18:0 (ANOVA, $F_{(3, 70)} = 3.5430$, $p < 0.05$) and 18:1 (n-9) (ANOVA, $F_{(3, 70)} = 5.5882$, $p < 0.01$) were significantly lower in oocytes of females sampled at Foundiougne during the wet season when compared to the other sampling sites and seasons (Table 1).

A monthly comparison revealed no significant differences in oocyte 18:0 fractions at all 3 sampling sites. However, 18:1 (n-9) was significantly lower in oocytes sampled at Joal (ANOVA, $F_{(2, 12)} = 6.6282$, $p < 0.05$) in March than in May, whereas in oocytes sampled at Foundiougne, the FA was significantly lower in October than in April (ANOVA, $F_{(7, 43)} = 3.3316$, $p < 0.01$; Figure 6.4).

The proportions of ARA were significantly lower in oocytes of females sampled at Joal than those sampled at Foundiougne during the wet season (ANOVA, $F_{(3, 70)} = 9.5558$, $p < 0.0001$). Further, oocyte ARA proportions at Djifer were significantly lower in February than in July (ANOVA, $F_{(4, 13)} = 5.9892$, $p < 0.05$). At this station, however, the proportions of EPA were significantly higher in July than in February (ANOVA, $F_{(4, 13)} = 4.8521$, $p < 0.05$). While monthly differences in DHA became apparent, these differences were not significant (Figure 6.5). The ratio of $\sum$ (n-3) to $\sum$ (n-6) FA was higher in oocytes of fish sampled at Djifer and at Foundiougne during the dry season when compared to oocytes sampled at Joal and at Foundiougne during the wet season, albeit not significantly. The FA 16:1 (n-7) constituted rather large averages of 5.7 to 7.0%, and 18:1 (n-7) accounted for 3.4 to 4.2%.

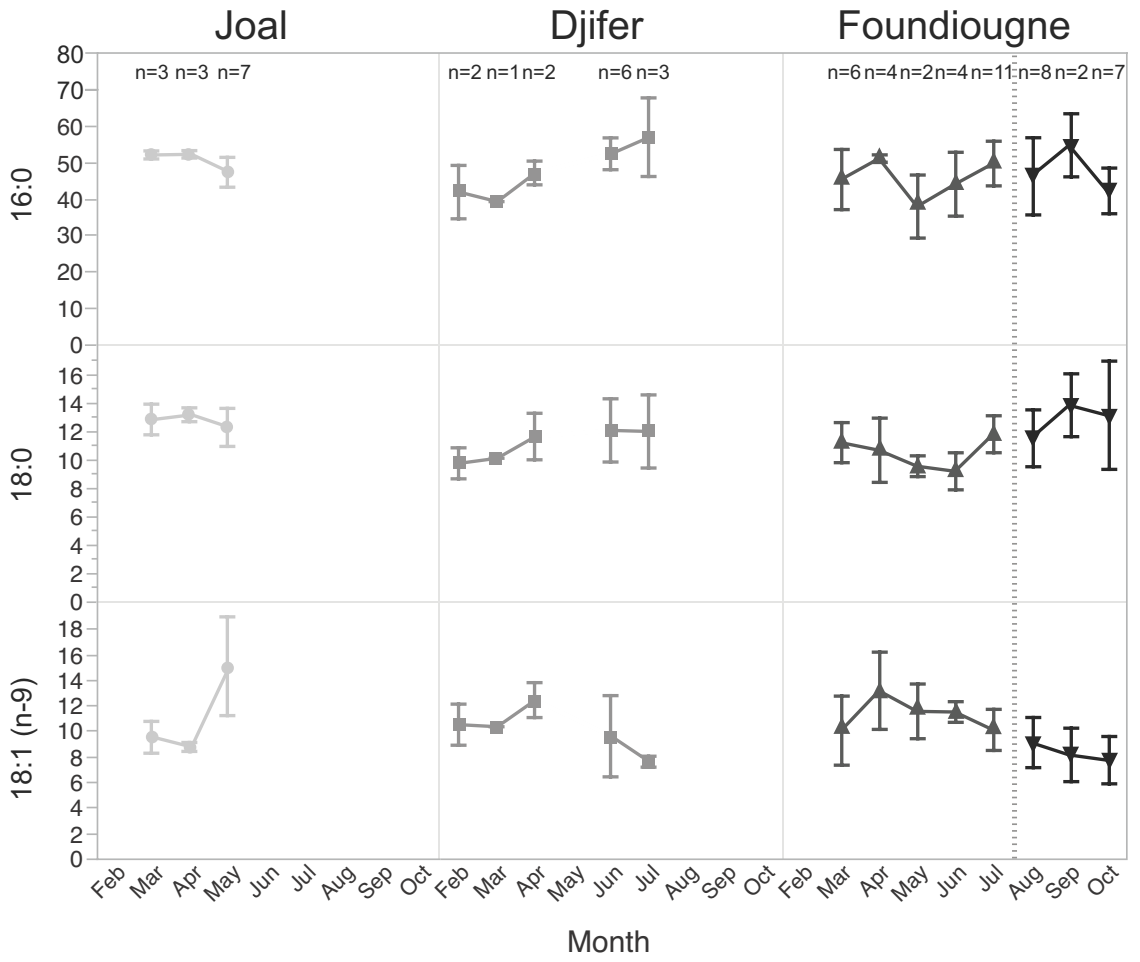

Figure 6.4 Monthly differences in the most abundant fatty acids 16:0, 18:0, and 18:1 (n-9) (as % of total fatty acids) (mean ± SD) in *Ethmalosa fimbriata* oocytes at the 3 sampling sites at the Senegalese South Coast (Joal) and inside the Saloum estuary (Djifer, Foundiougne) in 2014

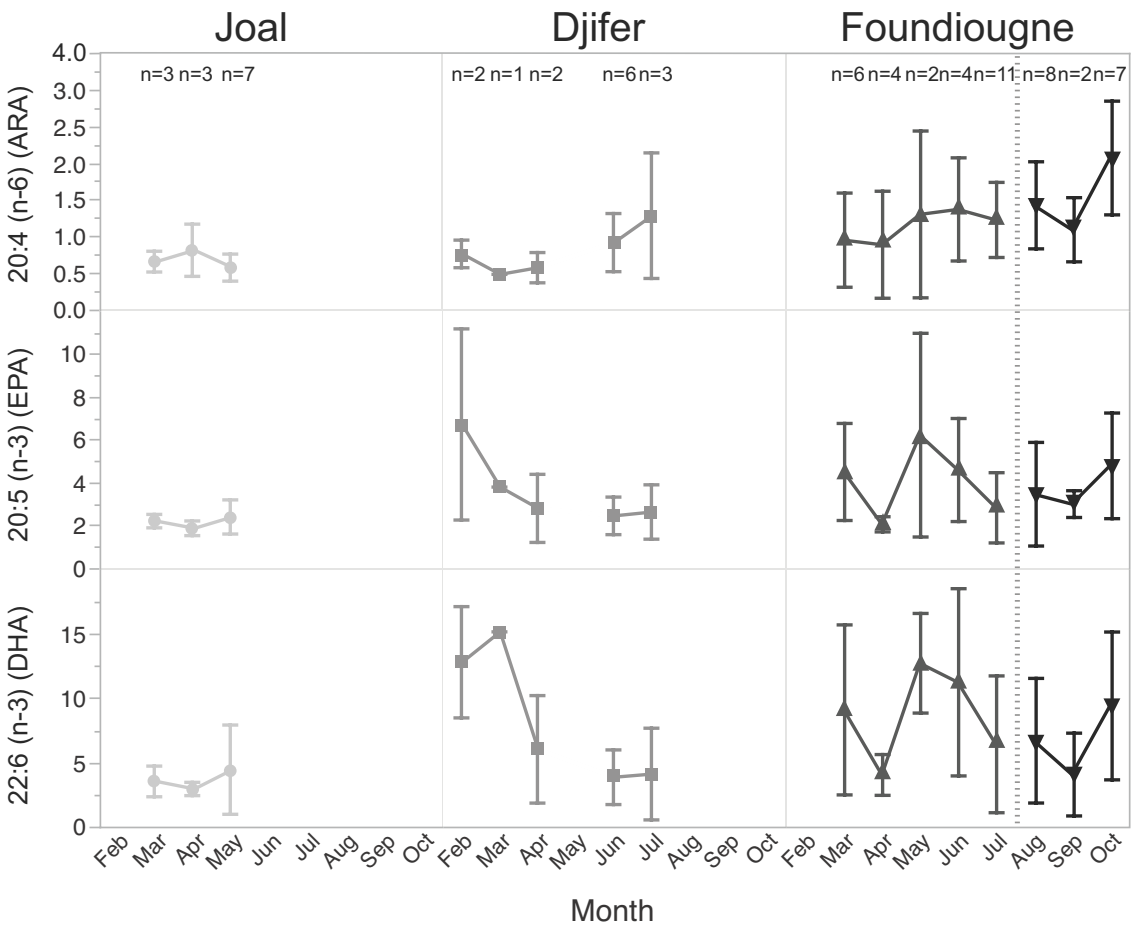

Figure 6.5 Monthly differences in the essential fatty acids 20:4 (n-6), 20:5 (n-3), and 22:6 (n-3) (as % of total fatty acids) (mean ± SD) in *Ethmalosa fimbriata* oocytes at the 3 sampling sites at the Senegalese South Coast (Joal) and inside the Saloum estuary (Djifer, Foundiougne) in 2014. ARA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid

6.3.4. Relationships between female reproductive investment, oocyte fatty acid compositions, and physical parameters

Results of the modeled relationships between oocyte FA compositions, female reproductive investment (*ODW*, W_O, *RBF*, I_G), and physical parameters are given in Table 6.2. A positive correlation between *RBF* and water temperature as well as salinity became apparent. *ODW* was negatively correlated with temperature and positively with salinity. The interaction of temperature and salinity, however, had a negative effect on *ODW*. Thus, under high salinity and high temperature conditions, females were producing higher numbers of oocytes albeit of lower dry weights.

The oocyte proportions of 18:1 (n-9) were positively correlated with W_{OF} and negatively with W_O (Table 6.2, Figure 6.6 a). Oocyte ARA fractions were positively correlated with surface water temperature and *RBF* (Table 6.2, Figure 6.6 b) Salinity had a negative quadratic effect on EPA, while the I_G of females had a positive and linear effect on this EFA (Table 6.2, Figure 6.6 c). Additionally, salinity had a negative and quadratic effect on DHA. The apex of the function was determined to occur at a salinity of 45 (Figure 6.6 d). Females spawning under intermediate salinity and high temperature conditions exhibited high ratios of ovary weight to body weight and high batch fecundities. These spawners were also able to invest higher proportions of examined EFA into their oocytes.

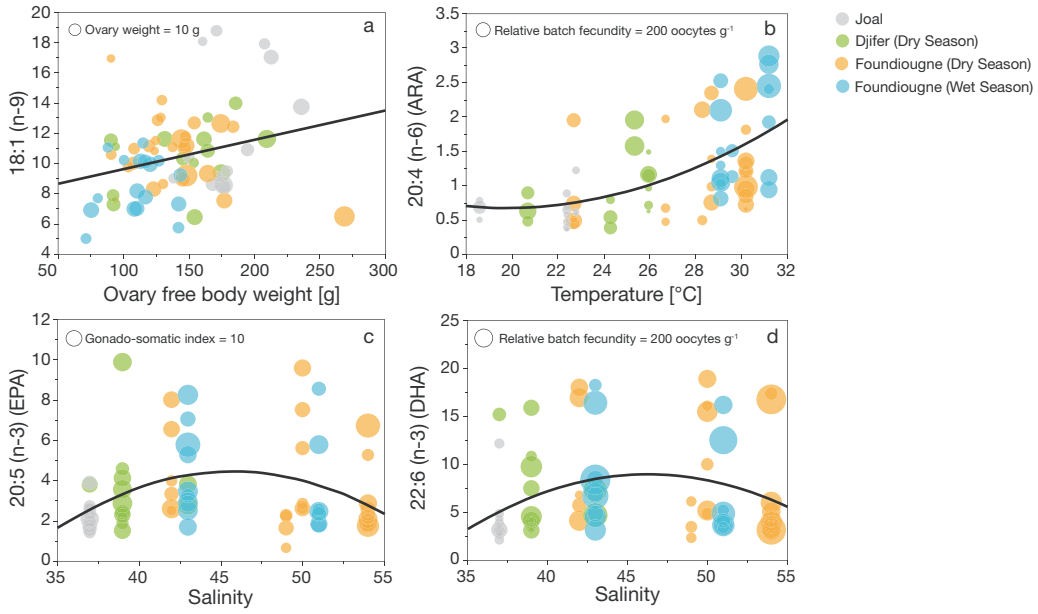

Figure 6.6 Relationships of 4 investigated fatty acids (as % of total fatty acids) with (a) female ovary-free body weight, (b) temperature, and (c, d) salinity. Respective bubble sizes display the ovary weights, relative batch fecundity, and gonado-somatic index of individual female *Ethmalosa fimbriata*. Different colours indicate the sampling location and season. ARA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid

6.3.5. Spatial and seasonal patterns in oocyte fatty acid compositions

PCA was used to investigate spatially and seasonally resolved differences in the most abundant FAs in the examined oocytes as well as EFA marker compositions. The first principal axis (PC1) explained 59.2% of the observed variability and mainly separated the sampling location of oocytes by their FA levels in 16:0 and 18:0, as well as ARA, EPA, and DHA.

FA levels of oocytes sampled at the Senegalese coast (Joal) were mainly described by negative PC1 loadings and thus by an increase in unsaturated FA. The second axis (PC2) separated oocytes according to the proportions of ARA and 18:1 (n-9) and explained 22.2% of the observed variance. The distribution of samples in the PCA plot showed strong overlaps between sampling sites/seasons. However, a distinct difference between the FA composition of oocytes sampled at Joal and Foundiougne during the wet season became apparent (Figure 6.7).

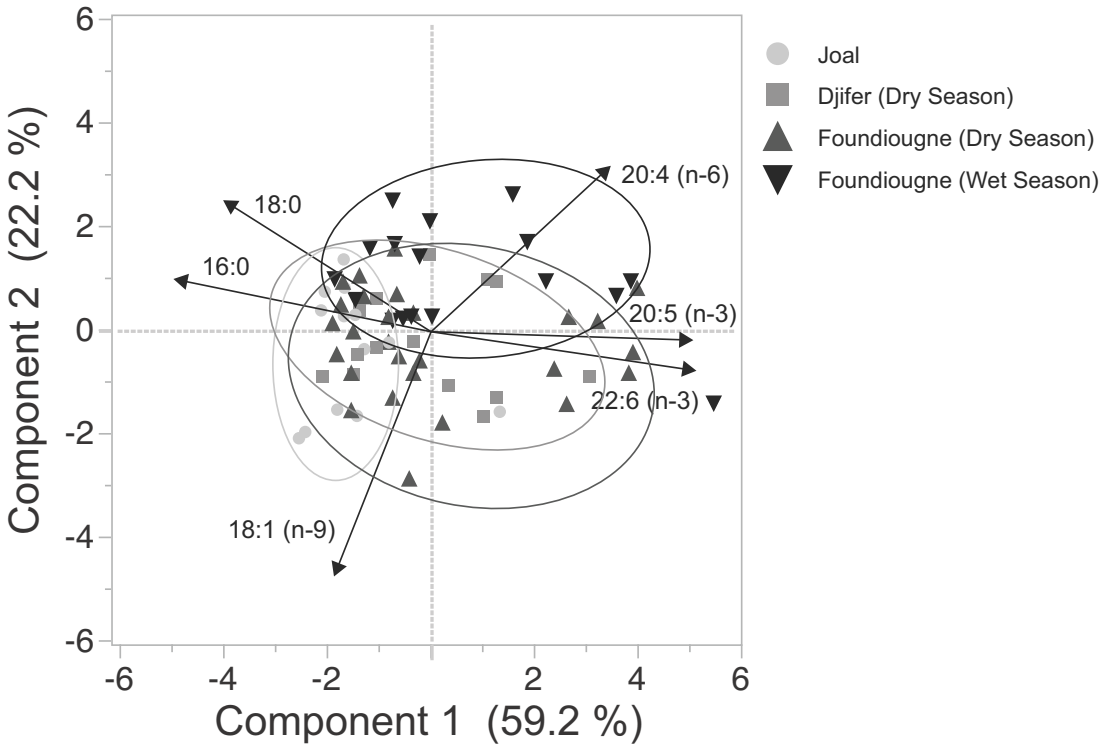

Figure 6.7 Principal component analysis on the most abundant fatty acids and essential fatty acids (% of total fatty acids) in *Ethmalosa fimbriata* oocytes sampled at Joal, Djifer (dry season), and Foundiougne (dry and wet season). Ellipses include 75% of the samples for each sampling site and season

Table 6.2 Results of the regression models for relative batch fecundity (*RBF*) oocyte dry weight (*ODW*), and fatty acids (FA) proportions in *Ethmalosa fimbriata* oocytes against temperature (*T*), salinity (*S*), ovary free body weight (W_{OF}), ovary weight (W_O), *RBF*, and gonado-somatic index (I_G). Estimates of the parameters for the least adequate models are shown, together with their respective correlation coefficients (R^2), standard errors (SE) and p-values. RMSE: root mean square error; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

	Relative batch fecundity			**Oocyte dry weight**			**18:1 (n-9)**			**20:4 (n-6) (ARA)**			**20:5 (n-3) (EPA)**			**22:6 (n-3) (DHA)**		
	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p
Intercept	-0.132	1.027	0.898	95.322	12.657	<0.0001	8.302	1.156	<0.0001	-2.080	0.386	<0.0001	0.707	0.428	0.103	0.940	0.520	0.075
T	0.159	0.061	0.012	-6.651	1.075	<0.0001				0.069	0.016	<0.0001						
S	0.027	0.027	0.324	2.464	0.418	<0.0001							0.005	0.010	0.658	0.124	0.012	0.054
T^2				0.755	0.199	0.0003												
S^2	-0.028	0.008	0.001	0.674	0.172	0.0002							-0.005	0.002	0.025	-0.007	0.003	0.011
T* x *S	0.042	0.016	0.010	-1.894	0.419	<0.0001												
RBF										0.001	0.001	0.008						
I_G													0.036	0.0141	0.012			
W_{OF}							0.034	0.009	<0.001									
W_O							-0.194	0.053	<0.001									
R^2		0.348			0.445			0.235			0.429			0.178			0.108	
RMSE		0.729			7.170			2.527			0.423			0.493			0.602	

6.3.6. Examination of trophic footprints

To evaluate a potential influence of site-specific differences in food items, the feeding history of *E. fimbriata* was investigated by analysing the FA composition of phytoplankton, female stomach contents, and oocytes at all 3 sampling stations at the end of wet season (November 2016). ARA (ANOVA, $F_{(2, 8)}$ = 13.2329, p < 0.01) and EPA (ANOVA, $F_{(2, 8)}$ = 17.4766, p < 0.01) were significantly lower in the phytoplankton sampled at Foundiougne than at Joal and Djifer (Fig. 8). DHA values were significantly lower in phytoplankton sampled at Foundiougne than at Joal, with intermediate values recorded at Djifer (ANOVA, $F_{(2, 8)}$ = 95.7254, p < 0.0001). The FA 16:1 (n-7) contributed an average of 7% to the phytoplankton total FA at Joal, whereas at Foundiougne this FA showed significantly higher proportions, varying around 12% (ANOVA, $F_{(2, 8)}$ = 6.7892, p < 0.05). The FA 18:1 (n-7) was, however, significantly higher in the phytoplankton sampled at Joal (4.7%) when compared to Djifer and Foundiougne, with mean values of 2.3 and 1.9%, respectively (ANOVA, $F_{(2, 8)}$ = 69.0017, p < 0.0001) (data not shown). No significant spatial differences in EFA composition nor in 16:1 (n-7) and 18:1 (n-7) could be determined in the stomach contents and oocyte compositions of females. However, especially at Foundiougne, distinct differences between the EFA proportions of phytoplankton, stomach contents, and oocytes became apparent (Figure 6.8).

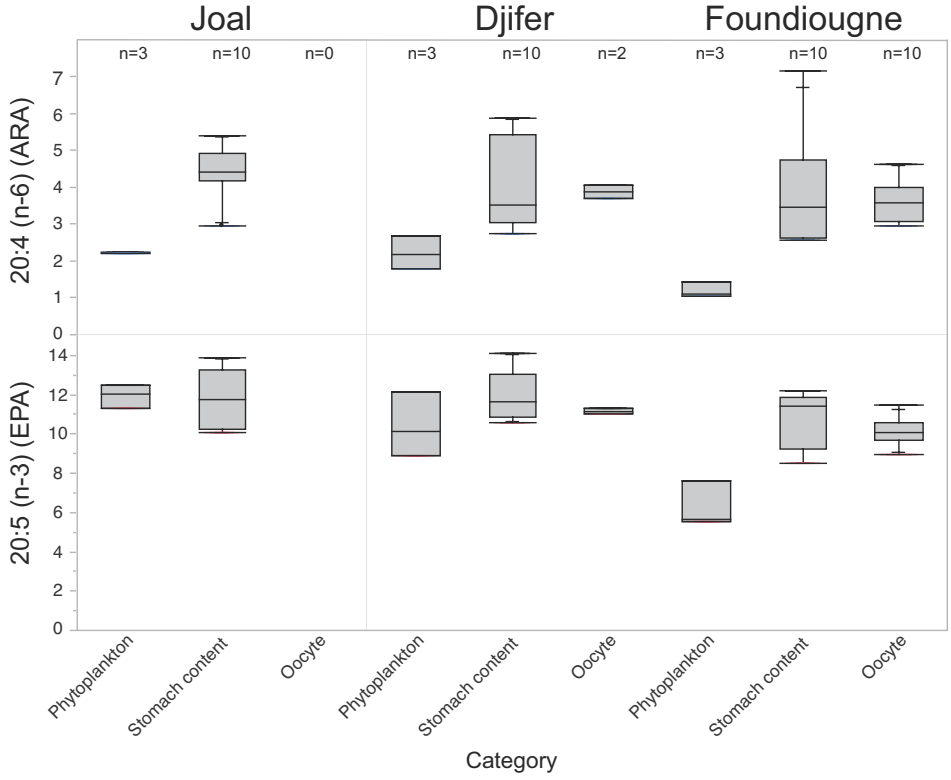

Figure 6.8 Proportions of 20:4 (n-6) and 20:5 (n-3) (as % of total fatty acids) in November 2016 for microphytoplankton in surface waters and for the stomach contents as well as oocytes of female *Ethmalosa fimbriata*. Box-and-whisker plots display the median (line), interquartile range (box), interdecile range (small whiskers), and the 5th and 95th percentiles (big whiskers). ARA: arachidonic acid; EPA: eicosapentaenoic acid

6.4. Discussion

The reproductive potential of a stock is strongly associated with female reproductive effort and gamete quality (Trippel 1999, Jakobsen et al. 2009). Egg quality, often taken as a measure for hatching success or viability of early larval stages (Morgan 2008), can be related to many factors: the size or weight of the oocyte (Blaxter & Hempel 1963); the amount of nutrients, lipids, and proteins deposited into the oocyte (Brooks et al. 1997); and also the fatty acid (FA) composition of the oocyte (Wiegand 1996). We investigated oocyte quality in terms of FA composition in relation to individual reproductive investment and how it is influenced by ambient temperature and salinity in a hypersaline estuary.

Observed water temperature and salinity variations in 2014 followed a seasonal pattern typical for the Sine Saloum estuary (Simier et al. 2004). Water temperature steadily increased until the end of the wet season in October. The highest salinity values were recorded in July, at the end of the dry season, and the lowest in September/October, at the end of the wet season. *Ethmalosa fimbriata* produced increasing numbers of oocytes, along a rising temperature and salinity gradient. However, the interaction of temperature and salinity exhibited negative effects on the weight of the hydrated oocytes. Results thus support the assertion that oocyte weight is negatively correlated with batch fecundity in clupeid fishes (Bradford & Stephenson 1992). OV, however, constituted a conservative spawning trait in *E. fimbriata* because no differences among sampling sites and seasons were detected. Further, heavier females incorporated more 18:1 (n-9) into their oocytes, even though higher ovary weights led to smaller fractions of this FA in the produced oocytes. (Peters et al. 2015) observed a negative correlation of 18:1 (n-9) proportions with larval length in European sprat *Sprattus sprattus* (Linnaeus, 1758) and consequently assigned this particular FA an important role as a storage lipid. Our findings illustrate a trade-off mechanism between ovary weight (number of produced oocytes) and maternal 18:1 (n-9) supply to individual oocytes. Accordingly, Peruvian anchoveta *Engraulis ringens* (Jenyns, 1842) produced smaller amounts of eggs, albeit of larger sizes and lipid contents, to enhance egg and larvae survival under conditions with low water temperatures and low prey abundances (Castro et al. 2009).

Essential fatty acid (EFA) ratios remain more or less constant across the first 3 trophic levels of the food chain (St. John & Lund 1996). Consequently, EFA profiles and ratios in lipid depots such as oocytes are directly affected by the diet composition of the spawner (Dalsgaard et al. 2003, Castro et al. 2010, Patterson & Green 2015). Monthly variability in oocyte FA composition and strong correlations of FA biomarkers like ARA, EPA, and DHA with water temperature and salinity suggest immediate dietary effects on oocyte quality (Leray et al. 1984, March 1993, Patterson & Green 2015). However, the observed high variances in oocyte FA composition in *E. fimbriata* might be due to migratory behavior. Diet to egg transfer of ARA takes 2 to 16 days in

batch-spawning fishes (Fuiman & Faulk 2013). Given the rather small geographical ranges in the Sine Saloum (15 to 45 km), *E. fimbriata* individuals may easily migrate during this timeframe into the estuary to spawn after feeding in the ocean. In general, spawning migration combined with short-interval batch spawning is regarded as an adaptation to take direct advantage of nutrients critical for offspring survival which are available at the spawning site (Fuiman & Faulk 2013). Conversely, feeding on phytoplankton with beneficial attributes (i.e. FA compositions) for the survival of early life stages at a certain location/time might also be a viable strategy in heterogeneous environments (Crawford et al. 1986). Accordingly, Spanish sardine *Sardinella aurita* (Valenciennes, 1847) stored energy in the form of lipids during the highly productive upwelling season, which was later (after five months) released via metabolism for gamete production (Fréon et al. 1997).

Further evidence for migratory movements between marine feeding and estuarine spawning grounds is provided by the results of the sampling campaign conducted in November 2016 (end of rainy season). At Joal and Djifer, the EPA proportions in female stomach contents and oocytes reflected the EPA values of the sampled phytoplankton. At Foundiougne, the deviation in EPA proportions in phytoplankton and stomach contents/oocytes can only be explained by feeding migration. Still, the fractions of ARA in the phytoplankton did not mirror the stomach contents of females at all sampling sites. We explain this result by the spatial–temporal variability (patchiness) in estuarine and coastal phytoplankton communities. It may also hint towards a certain selectivity in the species' feeding behavior. Phytoplankton at Joal was sampled close to the beach, explaining the higher observed fractions in the biomarker for organic detritus 18:1 (n-7) and lower fractions in the diatom biomarker 16:1 (n-7) when compared to estuarine samples.

E. fimbriata mainly feeds on diatoms and dinoflagellates (Bainbridge 1961, Blay Jr. & Eyeson 1982b), but literature on phytoplankton community composition in tropical hypersaline environments is scarce. The most dominant taxa under hypersaline conditions in a South African estuary were cyanobacteria, although a resilience to high salinities was also detected in diatoms (Nche-Fambo et al. 2015). The trophic marker for a diatom-based diet 16:1 (n-7) indeed constituted rather large averages in all examined oocytes (Dalsgaard et al. 2003). Biomarkers for cyanobacteria 18:3 (n-6) and heterotrophic organisms 20:1 (n-9) and 22:1 (n-11), on the other hand, were absent in our samples (Dalsgaard et al. 2003, Yang et al. 2016). DHA to EPA ratios in oocytes greatly exceeded 1 at all sampling sites, which indicates a dinoflagellate-based ecosystem (Dalsgaard et al. 2003). However, the small ratios (<0.5%) of dinoflagellate-specific biomarkers such as 18:4 (n-3) and 18:3 (n-3) in the sampled oocytes make a dinoflagellate-based diet highly unlikely (Kopprio et al. 2015, Peters et al. 2015). The FA 18:1 (n-7) and 18:1 (n-9) were found to be quite relevant in the investigated oocytes, suggesting a contribution from detrital material to the spawners' diet (Fahl & Kattner 1993). Overall, these findings agree with our microscopic observations and classify

the food of *E. fimbriata* as mainly consisting of diatoms and degenerated organic matter.

At lower temperatures, phytoplankton increase cell membrane PUFA levels to maintain a certain level of fluidity. Inversely, higher SAFA levels are required at higher temperatures to maintain membrane integrity (Guschina & Harwood 2006, Hixson & Arts 2016). This process is commonly referred to as homeoviscous adaptation (Sinensky 1974). In agreement with these findings, *E. ringens* eggs sampled in cold waters off the coast of Chile exhibited high PUFA levels, whereas eggs sampled in warmer waters were characterized by higher SAFA and MUFA values (Castro et al. 2010). Consumption of phytoplankton adapted to high water temperatures thus explains the generally high ratios of SAFA in our material from warm tropical waters when compared to FA profiles in eggs and larvae of temperate clupeid species (Tocher et al. 1985, Peters et al. 2015). Additionally, increased temperatures were generally negatively correlated with (n-3) PUFA but positively correlated with (n-6) PUFA in phytoplankton (Hixson & Arts 2016). Females spawning in middle reaches of the Saloum River were indeed able to equip their oocytes with significantly higher ARA fractions, and this particular EFA was positively correlated with water temperature. Apart from ARA, however, females spawning inside the estuary generally equipped their oocytes with higher EFA proportions as illustrated by PCA and their positive relationships with spawner reproductive investment and salinity.

Amino acids derived from yolk hydrolysis and ions, such as K^+, Cl^-, P_i, and NH_4^+, are the main components of fish oocyte osmolality (Finn et al. 2002, Lubzens et al. 2010). In general, osmoregulation in the embryo begins with the development of extrabranchial ionocytes, which are located in the entire integument, especially on the yolk sac (Alderdice 1988, Kaneko et al. 2002, Seo et al. 2015). Ionocytes are rich in Na^+/K^+-ATPase (McCormick 1993, Armesto et al. 2014) and have been shown to be the site of ion excretion in fish acclimated to hypersaline environments (Foskett & Scheffey 1982). Prostaglandins are lipid compounds that regulate electrolyte balance at several sites in fish cells, including the Na^+/Cl^- co-transporter and the Na^+/K^+-ATPase (Van Praag et al. 1987). Conversion of ARA to prostaglandin in gills and other tissues has been reported in several teleost fishes (Ogata et al. 1978, Henderson et al. 1985, Beckman & Mustafa 1992). Enhanced dietary intake of ARA thus increased prostaglandin production (Bransden et al. 2004) and thereby the resistance to hyperosmotic stress in a variety of fish larvae (Koven et al. 2003, Willey et al. 2003). Further, the ratios of (n-3) to (n-6) PUFA in oocytes sampled from Djifer and Foundiougne during the dry season were numerically higher than at Joal and at Foundiougne during the wet season, albeit not significantly. It has been shown before that elevated levels in the ratio of (n-3) to (n-6) PUFA in gill lipids of *Anguilla anguilla* enhanced their ability to maintain osmotic equilibrium in waters of different salinities at different temperatures (Thomson et al. 1977, March 1993). *E. fimbriata* larvae from Sine Saloum hatching from oocytes with higher ratios of ARA and higher

ratios of (n-3) to (n-6) PUFA are therefore more likely to develop normally and survive in hypersaline environments, as they show elevated osmoregulatory capabilities.

The results obtained in the present study highlight the importance of incorporating information on oocyte quality in terms of FA composition in the assessment of stock reproductive potential. Under the hypersaline conditions inside the Sine Saloum estuary (temperature >27°C, salinity ~46), individual *E. fimbriata* spawned not only more eggs (per g female) but also eggs of higher quality. Female reproductive potential is therefore determined to be highest when spawning occurs inside the estuary at the end of the wet season (September/October), when temperatures are high and salinities are less stressful due to freshwater input. These findings, however, partly contradict the current paradigm that oocyte quality is negatively correlated with batch fecundity in teleost fishes.

6.5. Conclusion

Past studies have shown that spawning stock biomass exhibits a limited capacity to predict recruitment. More promising might be the determination of physiological strength in reproduction products to estimate the survival potential of a stock. Maternal and environmental effects on recruitment play a significant role and have to be understood for a more effective stock management. Our results highlight the importance of considering differences in gamete viability with respect to their essential fatty acid composition. Observed differences in oocyte fatty acid profiles are linked to the individual's reproductive investment and to different environmental conditions. This variability likely impacts the survival probability of early life stages and thus the reproductive potential of species such as *Ethmalosa fimbriata*, which serves as an example for clupeid fishes reproducing under extreme conditions.

6.6. Acknowledgements

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CHAPTER III

7. SPAWNING ENERGETICS AND OTOLITH MICROCHEMISTRY PROVIDE INSIGHTS INTO THE STOCK STRUCTURE OF *ETHMALOSA FIMBRIATA*

Julian Döring, Carola Wagner, Maik Tiedemann, Patrice Brehmer, and Werner Ekau

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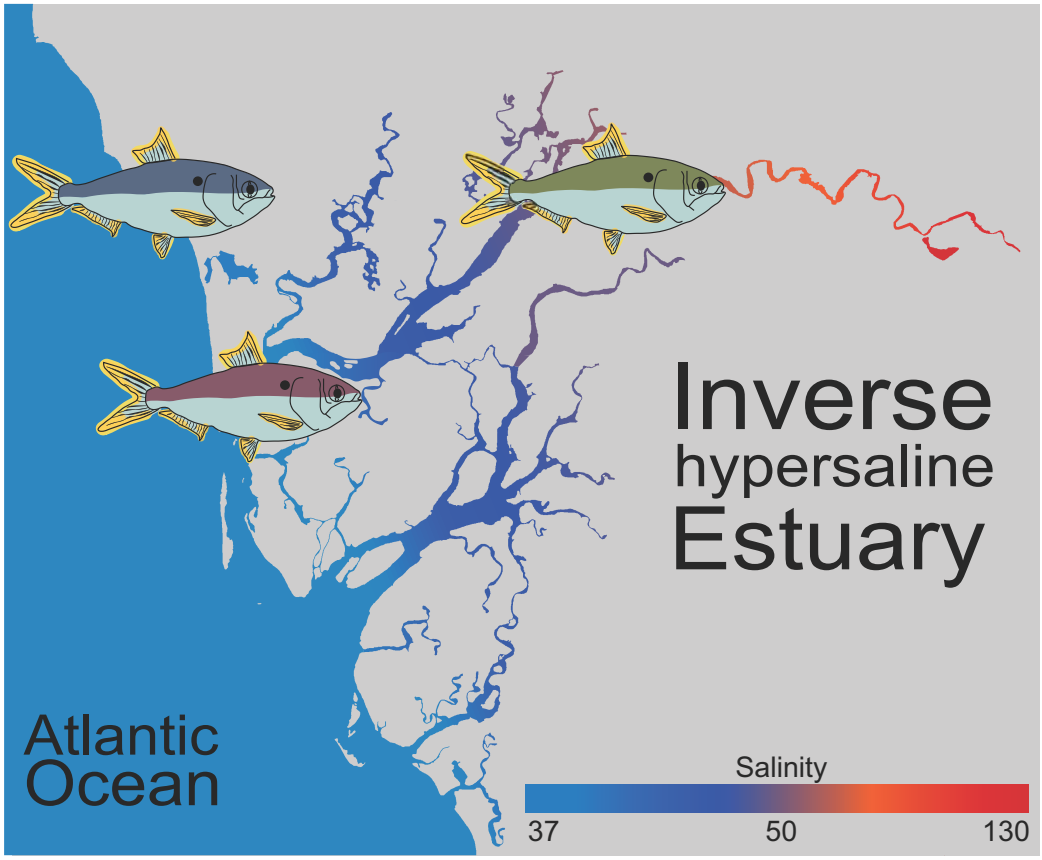
Inverse
hypersaline
Estuary
Atlantic
Ocean
Salinity
37
50
130

Abstract

Stock structure differentiation in marine fishes is the central pillar of population resilience to environmental variability and exploitation. A diverse population structure provides an insurance against recruitment failure and impacts of elevated fishing pressures. Accurate stock identification is, therefore, a prerequisite for deciphering the complexities responsible for recruitment variation. Not much is known, however, about the stock structure of bonga shad *Ethmalosa fimbriata* in Senegalese waters even though the species is one of the region's economically most important clupeid fishes. To identify different stock spawning components, the gross energy contents of spawning batches (fecundity, oocyte dry weight, oocyte lipid, and protein content), and the microchemistry of sagittal otoliths in individual female *E. fimbriata* were compared between contrasted sampling sites at the Senegalese south coast and inside the Sine Saloum estuary. Our results show that females spawning in the estuary's middle reaches invested almost three times more energy into reproduction (115 ± 65 J g^{-1} spawner) than their oceanic counterparts (39 ± 34 J g^{-1} spawner). Also, female otolith levels in Ba:Ca, Sr:Ca, and Zn:Ca either differed significantly between study sites or could be linked to heterogeneous environmental parameters. A linear discriminant analysis combining spawning energetics and otolith microchemistry yielded high classification percentages and thus evidenced distinct stock spawning components of *E. fimbriata* in Senegalese waters. Since less productive components are more likely to be overexploited, we suggest consideration of distinct ecosystemic management approaches for *E. fimbriata*'s stock. A further decline in the species' catch rates could compromise this fisheries' activity and thus affect the elaborate socio-economic sector that depends on it.

Keywords: Clupeidae · Stock spawning components · Inverse estuary · Reproductive investment · Oocyte

7.1. Introduction

The bonga shad (*Ethmalosa fimbriata,* Bowdich 1825) is an exploited clupeid fish, which occurs along the West African coast from Mauritania to Angola (Charles-Dominique & Albaret 2003). In Senegal, the species' fisheries landings totalled almost 17,000 t in 2014, making it one of the region's economically most important clupeid fishes (Ndaw et al. 2015). Former studies lead to the assumption that *E. fimbriata* is locally adapted in regard to its morphology, growth, and reproductive traits (Charles-Dominique & Albaret 2003). Observed differences in life history patterns indicate that West African populations are largely separated and only a few individuals are migrating between habitats (Charles-Dominique 1982, Panfili et al. 2006). Accordingly, a study by Durand et al. (2013) identified genetic differences between specimens sampled in West African oceanic waters (Guinea, Banc d'Arguin in Mauritania) and individuals sampled inside estuaries (Sine Saloum delta, Gambia, and Casamance Rivers). The authors attributed heterogeneous genetic patterns to alternating life cycles and to the species' occurrence in estuaries with distinct hydrological features. Still, in this study, no individuals from southern Senegalese shelf waters were sampled.

Especially in Senegalese coastal waters, *E. fimbriata* experiences several hydrological challenges. The species inhabits cold coastal upwelling waters as well as extremely warm waters in the Sine Saloum, a mangrove estuary severely affected by long-term climatic changes (Charles-Dominique 1982, Panfili, Durand, et al. 2004). Until the 1970s, this estuary used to be species-rich and supported a high-yielding fishery. Due to a decline in precipitation and rising temperatures in the Sahel zone over the past decades, this estuary transformed into an inverse system, with a permanently reversed salinity gradient (Pagès & Citeau 1990). The hypersaline conditions in the upper reaches of the estuary (salinity up to 130), as well as strong fishing pressure already led to an immense reduction in the overall annual catch rates of around 50 - 80% over the past 50 years (Villanueva 2015). As effective fisheries management requires assessment of connectivity patterns among populations, small-scale studies on the migration and dispersal of *E. fimbriata* in Senegalese waters are necessary to manage its stock more efficiently (Durand et al. 2013).

The term "stock" refers to a population that is reproductively self-sustaining and comprises individual fish displaying identical life history traits as a response to environmental parameters (Ihssen et al. 1981). Since certain traits may be expressed differently in the same genotype, as an adaptive response to environmental factors, stock discrimination solely on the basis of genetics renders insufficient (MacLean & Evans 1981, Begg et al. 1999, Swain & Foote 1999). Small pelagic fish stocks are often composed of different spawning components, and each component's contribution to the fished stock varies with its productivity (Begg et al. 1999). Generally, less productive components are more vulnerable to fishing pressure than more productive ones (Jennings et al. 1998). Thus, less productive components are more likely to be

overexploited, which ultimately leads to a loss of genetic variability (Stephenson 1999). To facilitate stock assessment accuracy and to improve fisheries management plans, the relative contribution of a spawning component to the entire stock needs to be estimated. Thus, recruitment and the reproductive potential need to be determined for each component separately to avoid incorrect estimates of stock size and productivity (Hilborn 1985).

Female reproductive potential changes due to heterogeneous environmental conditions at the spawning sites through shifts in energy allocations (Pecquerie et al. 2009). The entire stock's productivity is therefore subject to variations in reproductive potential of individual females which in turn is subject to the surplus energy available for spawning (Döring et al. 2018a). Hatching success or viability of early larval stages was found to be related to maternal investments, such as the weight of the oocyte and the amount of lipids and proteins deposited into the oocyte (Brooks et al. 1997, Kamler 2005). An increase in egg lipid and protein content enhances hatching success in clupeoid fishes (Castro et al. 2009). Further, elevated energy reserves (protein and lipid contents) support larval condition by increasing the time window until first feeding has to be established (Guisande et al. 1998). Thus, knowledge on egg composition is essential, since it has direct consequences on early life stage survival. The number of viable offspring is also influenced by the number of produced oocytes i.e. the batch fecundity in indeterminate spawners such as *E. fimbriata* (Rickman et al. 2000). An individual female's ability to invest energy into reproduction is therefore closely coupled with the stock's reproductive potential and with its productivity (Pecquerie et al. 2009).

Apart from determining a stock's productivity the identification of spawning areas and migration patterns are crucial to develop sustainable fisheries' management strategies (Colloca et al. 2009). Preservation and management of spawning areas strengthen the stability of fished resources and thus avoid their decay to irreversible degrees (Stephenson 1999, Avigliano et al. 2017). A decline in catch rates could further compromise the fisheries' continuity and thus affect the wide-ranging socio-economic sector that depends on it (Beck et al. 2001). In order to identify spawning areas and to discriminate among stock components, elemental analysis of fish otoliths is a widely used methodology (e.g. Kerr & Campana 2013, Avigliano et al. 2017). The chemical composition of otoliths is a natural marker of habitat use due to the otolith's continuous growth throughout the fish's lifetime and its metabolic inertness (Campana & Neilson 1985). Physiological factors, as well as variations in water chemistry, can affect the incorporation of trace elements in the otolith (Campana 1999). Accordingly, it has been shown that the predominant source of the incorporated elements Strontium (Sr) and Barium (Ba) is the surrounding water (Bath et al. 2000). Soft acid metal ions such as Zink (Zn), on the other hand, may mainly originate from the diet (Ranaldi & Gagnon 2008). Therefore, the concentration of selected elements (the "elemental fingerprint") in the otolith can be used as a natural tag to discriminate between groups of fish that

have spent at least part of their lives in different environments (Kerr & Campana 2013).

This study aims at assessing the stock structure of *E. fimbriata* in Senegalese coastal waters based on spawning energetics/productivity in individual females and their otolith elemental fingerprint. For this purpose, the gross energy contents of spawning batches (taking into account fecundity, oocyte dry weight, oocyte lipid and protein content) and the microchemistry of sagittal otoliths were compared between one sampling site at the Senegalese coast (Joal), one at the estuary's mouth (Djifer) and one inside the Sine Saloum estuary (Foundiougne).

7.2. Materials & Methods

7.2.1. Study area

The Senegalese South Coast (SSC; 14°36'N to 13°36'N) is located in the southern part of the Canary Current Large Marine Ecosystem, one of the most productive ecosystems in the world. The SSC harbours a seasonal upwelling cell, which governs ambient temperature fluctuations in the region. Because of the shelf's topography, northerly trade winds induce a strong tongue-shaped upwelling core in winter/spring (Capet et al. 2017, Ndoye et al. 2017). While in coastal regions with a narrow shelf the upwelling core is observed at the shelf break, the upwelling core at the SSC occurs on the shelf, where water depths are shallower than 100 m (Arístegui et al. 2009, Ndoye et al. 2014). In concert with sunlight, the upwelled cold and nutrient-rich bottom waters facilitate the growth of phytoplankton which constitutes the basis of marine food webs (Auger et al. 2016).

The Sine Saloum estuary is located at the southern tip of the SSC and extends over an open water surface of approximately 800 km² (from 13°55 and 14°10 N to 16°03' and 16°50' W; Figure 7.1). This coastal ecosystem is characterized by slight freshwater input due to isolated groundwater deposits and small inflow of rivers, while rainfall is the main freshwater supply in the system. Its river system consists of three main branches: the Saloum, Diomboss, and Bandiala. The water column of these main branches is well mixed and reaches a maximum depth of 15 m (Sloterdijk et al. 2017). The climate in the Sine Saloum region is characterized by a dry season (usually from November to June) and a short warm rainy season (usually from July to October) (Pagès & Citeau 1990).

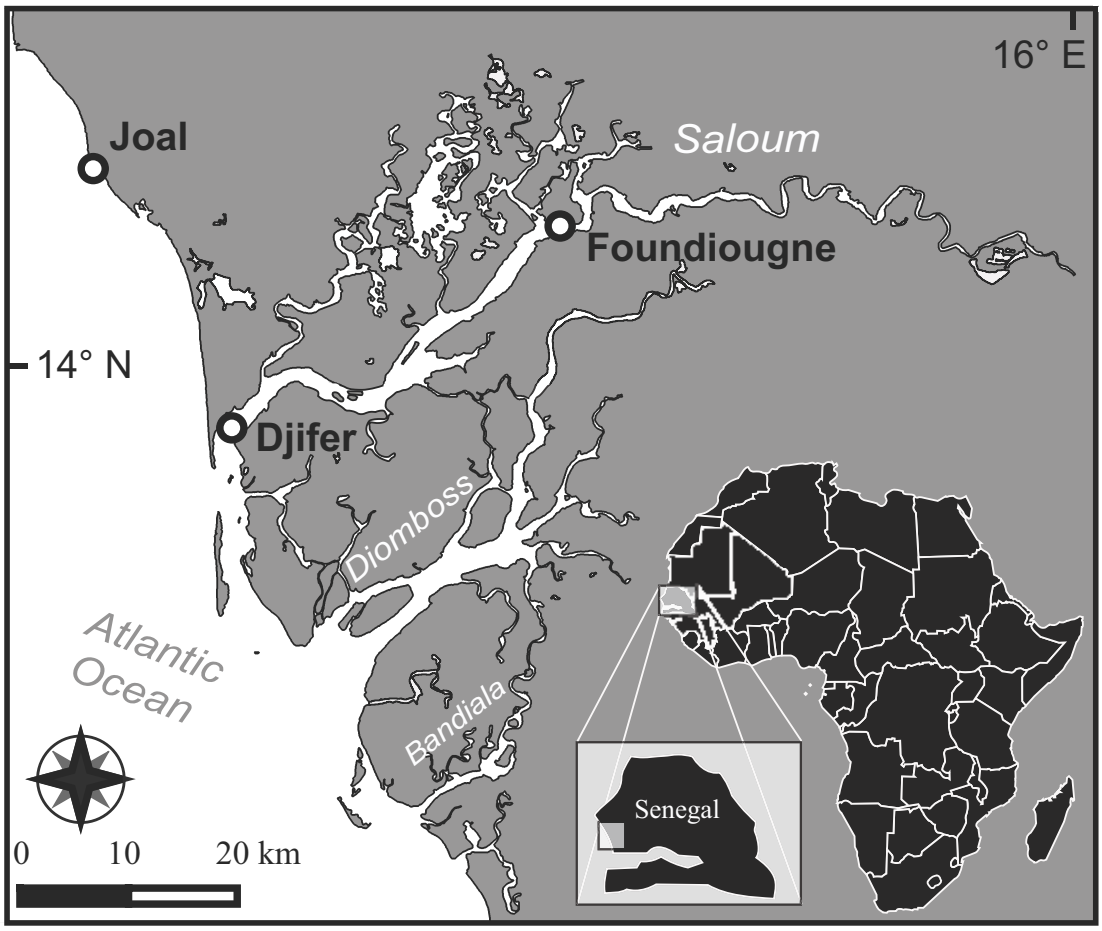

Figure 7.1 Map of the Senegalese south coast and the Sine Saloum estuary, including sampling sites: Joal (Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches)

7.2.2. Environmental parameters

Satellite-derived (Moderate-resolution Imaging Spectroradiometer – MODIS Aqua, level 2, 0.1 degrees) sea surface temperatures were assessed at Joal (15 km radius) and Djifer once per sampling week. As no remote sensing data for inland waters are available, the Saloum River's surface water temperatures were recorded *in-situ* once per sampling week with a digital thermometer (ama-digit ad 15 th; precision 0.4%; accuracy 0.4%). At Djifer and Foundiougne, salinity was determined once per sampling week according to the Practical Salinity Scale (PSS-78) with a handheld refractometer (Aqua Medic; precision 0.7%, accuracy 0.2%) using *in situ* water samples. For Joal, monthly means in MODIS satellite-derived (Aquarius, level 3, 0.5 degrees) sea surface salinities were used.

7.2.3. Sample collection

Monthly sampling was conducted at the Senegalese south coast and inside the Saloum River from February to October 2014, during *Ethmalosa fimbriata*'s extended spawning season (Charles-Dominique 1982, Panfili, Durand, et al. 2004). Three environmentally contrasted study sites were chosen: Joal (Senegalese South Coast, 14°9.1' N; 16°51.7' W), Djifer (Saloum River's mouth, 13°57.8' N; 16°44.8' W), and Foundiougne (Saloum River's middle reaches, 14°8.1' N; 16°28.1' W) (Figure 7.1).

Fish were caught with gill nets (32 - 36 mm mesh size) by local fishermen and immediately stored on crushed ice after landing. Approx. 1000 fish per sampling site and month were examined randomly in order to find stage V females, i.e. mature individuals with ovaries containing fully hydrated oocytes (Blay Jr. & Eyeson 1982a). Sagittal otoliths were extracted, rinsed with ethanol (70%), and stored dry in Eppendorf caps. Females that spawned recently or lost part of their egg batch during handling were rejected.

7.2.4. Spawning batch energy content

Total wet weight (W_W±0.01 g) and total length (L_T, nearest mm) were obtained from individual stage V females. Ovaries were dissected, and oocytes were extracted out of one ovary lobe, rinsed with deionized water, and counted under a stereomicroscope. Around 80 hydrated oocytes per fish (ca. 70 for lipid analysis, ca. 10 for protein analysis) were transferred to a pre-weighed tin cap, stored in cryovials, and deep-frozen in liquid nitrogen. Dissected ovaries were transferred to a 4% borax buffered formaldehyde and freshwater liquid for fecundity analysis. Absolute batch fecundity (*ABF*) was estimated gravimetrically using the hydrated oocyte method for indeterminate spawners (Hunter et al. 1985). The female's relative batch fecundity (*RBF*) was calculated by dividing *ABF* with the ovary free body weight (W_{OF}; Alheit 1988) (for details see CHAPTER I).

Oocytes in tin caps were freeze-dried (24 h) and weighed again to ascertain their dry weights (*ODW* ± 0.1 µg). Oocyte lipid content was determined by gas chromatography flame ionization detection (GC-FID). Three random samples were processed via GC mass spectrometry to ensure that all lipid classes were detected by the GC-FID. The total lipid content was assessed via summation of the weights of all detected fatty acids (for details see CHAPTER II). For protein analyses, counted oocytes in tin caps were dried at 40°C for >24 h and weighed again for dry weight determination. Total organic carbon (C) and nitrogen (N) content was measured using a EuroVector EuroEA3000 Elemental Analyzer. From the total amount of N in the sample, the protein content was calculated according to Kjeldahl (Bradstreet 1954), using a nitrogen-protein conversion factor of 6.25. Oocyte lipid content was determined for 57 females, whereas oocyte protein content was measured for 67 females.

The oocyte gross energy content (J) was calculated on the basis of measured protein and lipid content, which were multiplied by corresponding energy values from literature: The amount of proteins per given oocyte (P, mg) was multiplied by a factor of 23.66 J mg^{-1} and subsequently added to the total amount of lipids per oocyte (L, mg) multiplied by 39.57 J mg^{-1} (Henken et al. 1986). Dividing the oocyte's energy content by the oocyte's dry weight allowed for calculation of the oocyte's calorific value (J mg^{-1}).

The oocyte energy content of each individual *E. fimbriata* female was multiplied by its respective relative batch fecundity (*RBF*) in order to obtain a standardized estimate of the total amount of energy invested into a single spawning batch per unit body weight (*SBEC*, J g^{-1} W_{OF}) (Equation 7.1) (Döring et al. 2018a). The *SBEC* was calculated for 52 females.

$$SBEC = \left[\left(\mathrm{P} \times 23.66\ \frac{\mathrm{J}}{\mathrm{mg}}\right) + \left(\mathrm{L} \times 39.57 \frac{\mathrm{J}}{\mathrm{mg}}\right)\right] \times RBF \qquad (7.1)$$

7.2.5. Otolith elemental analyses

Dried sagittal otoliths were embedded in epoxy resin (Araldite 2020; Huntsman, USA) on glass slides. They were ground from the proximal side down to the nucleus using an MPS2 surface-grinding machine (GN, Nürnberg, Germany) and polished with a diamond-grinding wheel (grain size 15 µm). Concentrations of eight elements (Mg, Mn, Cu, Zn, Sr, Y, Ba, and Pb) were determined along transects of up to 2000 µm length along the rostrum's anterior edge on the otolith's proximal side. Analyses were carried out by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) using a NewWave UP193 solid-state laser coupled to a Thermo-Finnigan Element2 ICP-MS. The employed analytical procedure used a pulse rate of 10 Hz, an irradiance of ca. 1 GW cm^{-2}, a spot size of 75 µm, and an ablation speed of 3 µm s^{-1} (for details see Marohn et al. 2011). For external calibration the glass reference material NIST610 was analyzed after every second ablation path, using reference values of Jochum et al. (2011). Data quality was validated by analyzing a pressed pellet of NIES22 otolith (Yoshinaga et al. 2000) as well as through regular analyses of BCR-2G and BHVO-2G standard glasses (Jochum et al. 2005). For standard measurement results see Appendix Table B. Precision was <2% and the accuracy was <6% for Zn. To account for the substitution of Calcium (Ca) by the divalent elements Ba, Sr, and Zn all element concentrations are given as element:Ca ratios (Campana 1999). Elemental analysis was conducted on the otoliths of 30 female fish and mean otolith Ba:Ca, Sr:Ca, and Zn:Ca ratios were employed in order to discriminate between spawning sites. Differences in these element:Ca ratios are commonly examined in studies on shad migration behaviour and stock delineation (e.g.

Limburg 1995, Secor & Rooker 2000, Magath et al. 2013, Avigliano et al. 2017, Rohtla et al. 2017).

7.2.6. Statistical analyses

One-way analysis of variance (ANOVA) accompanied by Tukey honest significant difference (HSD) post hoc tests was carried out to test for significant differences in oocyte energy content, oocyte calorific value, spawning batch energy content between sampling months and sampling sites. ANOVA was also used to test for spatial differences in elemental concentrations (Ba:Ca, Sr:Ca, and Zn:Ca). Previously, normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) was tested. Data on oocyte energy content did not meet the homogeneity assumption and was square root transformed. The bivariate relationships between, oocyte lipid/protein content and ODW, as well as between element:Ca, temperature, and salinity were tested for significance using linear regression analysis. To test for direct effects of environmental parameters on the otolith element:Ca concentrations, the mean values of the last 100 µm of the traverse (otolith edge) were applied in linear regression analysis. Female length did not pose as a significant predictor for any of the tested variables. Outliers were identified using the outlier boxplot function in JMP 10.0.1 (SAS Institute Inc., Cary, NC, www.jmp.com) The significance level for all tests was set to 5%. All mean values are given ± standard deviation (SD).

A linear discriminant function analysis (LDFA) was performed to obtain the cross-classification matrix and to determine the capacity of these variables to identify the spawning site of the sampled females. The expected classification accuracies were calculated based on chance alone given the number of groups and sample sizes. Because of small sample sizes, Pillai's trace test was used to determine if the classification success rate was significantly different from random. Multicollinearity between mean element:Ca ratios was analyzed using variance inflation factor (VIF <2), a false outcome in the LDFA and the use of redundant variables in the study were therefore averted. The accuracy of the final predictive model was assessed using Leave-one-out-cross-validation. The 22 individual females for which all parameters (oocyte calorific value, *SBEC*, Ba:Ca, Sr:Ca, and Zn:Ca) could be assessed were used as input for the discriminant analysis.

7.3. Results

7.3.1. Female length distributions

Females with ovaries containing hydrated oocytes were sampled in the size ranges 23.1 - 27.7 cm, 20.8 - 26.9 cm, and 20.1 - 28.3 cm, at Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum River's middle reaches), respectively. Within the observed distributions, the modes of the highest frequency ranged around 25.5 cm at Joal and 23.5 cm at Djifer and Foundiougne (Figure 7.2).

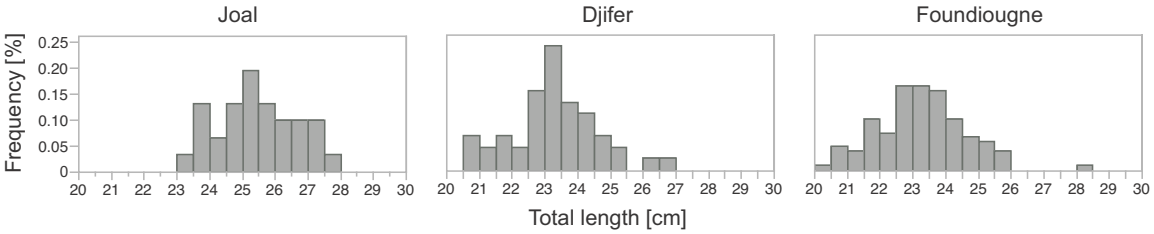

Figure 7.2 Length frequency distribution of hydrated *Ethmalosa fimbriata* females. Sampling took place at the Senegalese South Coast (Joal), at the Saloum River's mouth (Djifer), and inside the river's middle reaches (Foundiougne) from February to October 2014

7.3.2. Spawning energetics

Mean absolute oocyte lipid content was determined to be 0.9 ± 0.5 µg, corresponding to 2.7 ± 1.3% of oocyte dry weight. A mean protein content of 22.2 ± 7.5 µg or 64.6 ± 5.0% of the oocyte dry weight was recorded. Linear relationships between oocyte absolute lipid content and *ODW* ($F_{(56)} = 38.6$, $p < 0.0001$; $r^2 = 0.41$, Figure 7.3 a), as well as between oocyte absolute protein content and *ODW* were identified ($F_{(66)} = 301.1$, $p < 0.001$, $r^2 = 0.82$, Figure 7.3 b).

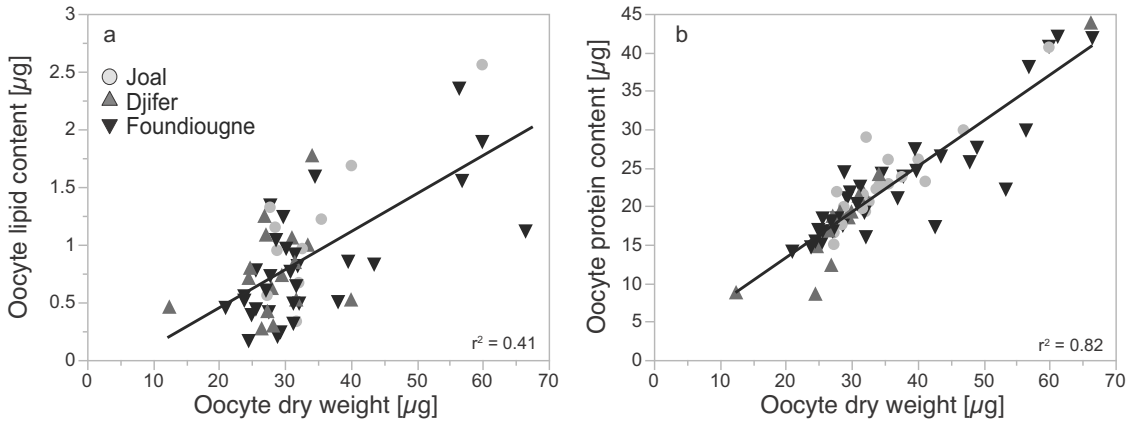

Figure 7.3 Relationships between dry weight, protein content (a), and lipid content (b) of *Ethmalosa fimbriata* oocytes sampled from females in Senegalese coastal waters. Specimens were sampled at the Senegalese South Coast (Joal, light grey dots), at the Saloum River's mouth (Djifer, dark grey triangles), and inside the river's middle reaches (Foundiougne, black triangles)

The energy contents of single oocytes spawned at Foundiougne (Saloum River's middle reaches) were significantly different among sampling months (ANOVA $F_{(7, 29)} = 6.4916$, $p < 0.001$). Oocyte energy content was significantly higher in March through May than in October 2014 (Tukey HSD $p < 0.05$). No monthly differences in oocyte energy content were observed at Joal (ANOVA $F_{(2, 12)} = 1.6024$, $p = 0.2491$) and at Djifer (ANOVA $F_{(3, 9)} = 1.1254$, $p = 0.4106$). Further, no significant spatial or temporal differences in oocyte calorific values were detected. The mean value across all sampling sites was estimated to be 15.2 ± 1.4 J mg^{-1}.

Also, no significant monthly differences in energy invested into one spawning batch (*SBEC*) were detected. Still, females sampled at Foundiougne invested significantly more energy into spawning when compared to their counterparts sampled at Joal (ANOVA $F_{(2, 51)} = 8.9255$, $p < 0.001$; Tukey HSD $p < 0.05$; Figure 7.4).

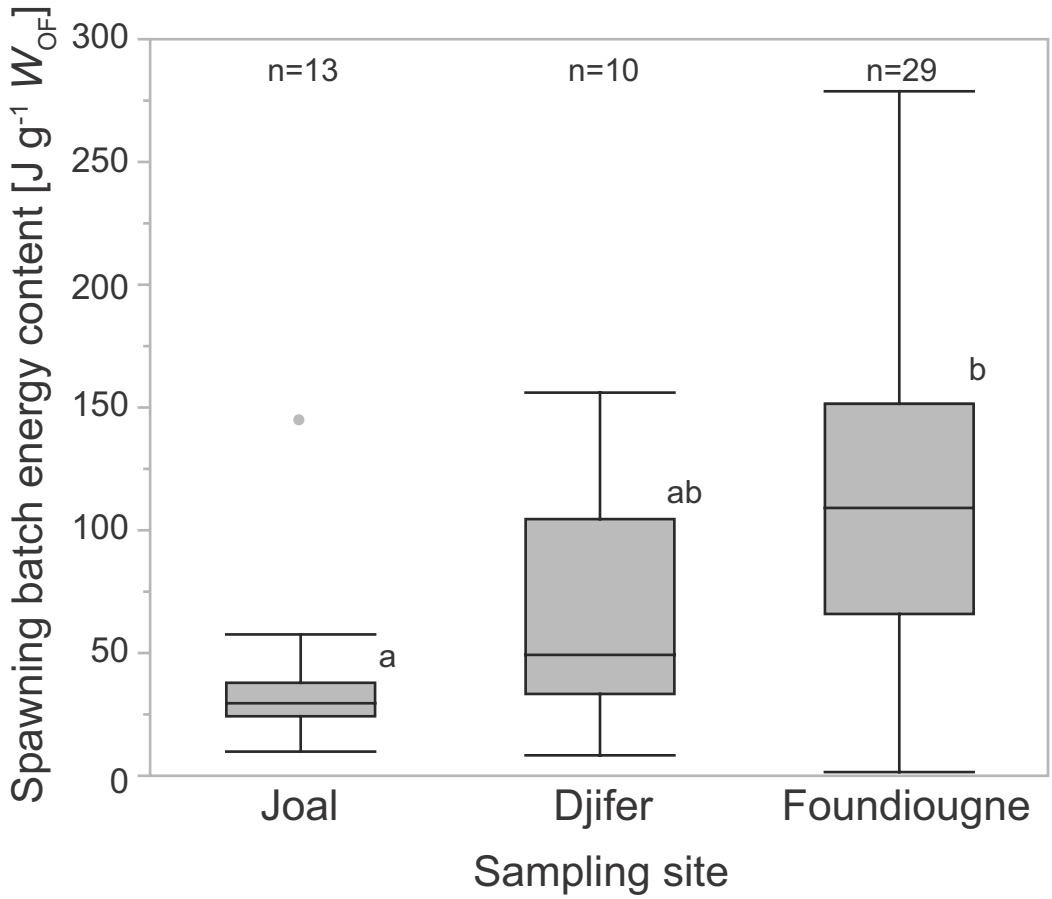

Figure 7.4 Spawning batch energy content of female *Ethmalosa fimbriata* sampled at the Senegalese South Coast (Joal), at the Saloum River's mouth (Djifer), and inside the Saloum River's middle reaches (Foundiougne). Whiskers show the 5th and 95th percentiles, solid lines indicate median values, and outliers are marked as dots. Differences between stations were significant if respective boxplots do not share the same letter (Tukey HSD). W_{OF}: Ovary free body weight

The LDFA plot employing the seasonally unaltered reproductive traits oocyte calorific value and *SBEC* (Figure 7.5 a) showed little separation between sampling sites. The LDFA cross-classification matrix (Table 2) revealed a moderate to low percentage of correctly classified individuals for Joal (83.3%), Djifer (20.0%), and Foundiougne (72.7%), although not significantly different from random (Pillai's trace test: p = 0.1053), providing no discriminatory power.

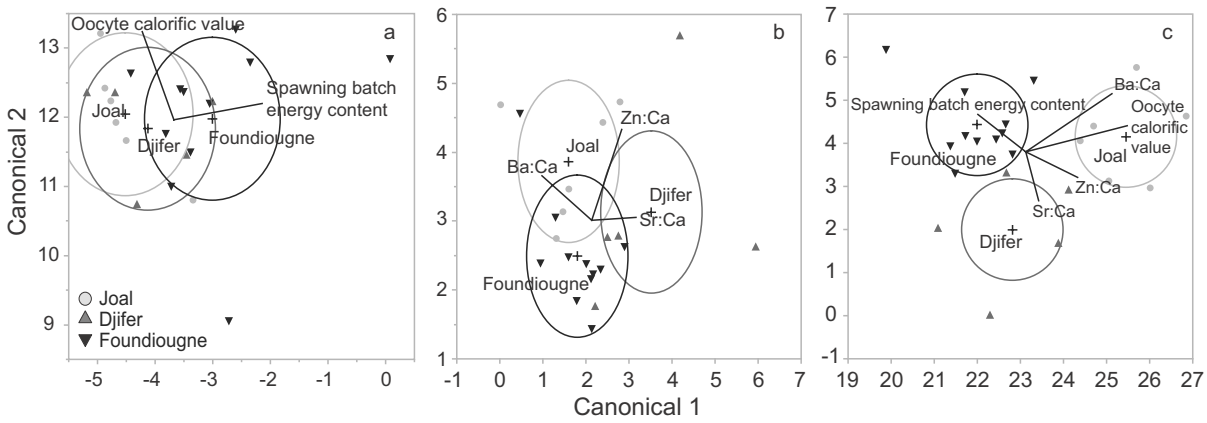

Figure 7.5 Linear discriminant function analysis of the spawning energetics (a), otolith element:Ca ratios (b), and both techniques combined (c) in female *Ethmalosa fimbriata* sampled at the Senegalese South Coast (Joal, light grey dots), at the Saloum River's mouth (Djifer, dark grey triangles), and inside the river's middle reaches (Foundiougne, black triangles). Ellipsoids include ca. 50% of a sampling station's data points

7.3.3. Otolith microchemistry

Otolith mean values in Ba:Ca throughout the entire lifetime of the female fish were significantly higher in individuals sampled at Joal than at Djifer and Foundiougne (ANOVA, $F_{(2, 27)} = 7.5$, $p < 0.01$). Also, otolith mean Zn:Ca values were significantly higher at Joal than at Foundiougne, with otoliths of females sampled at Djifer exhibiting intermediate values (ANOVA, $F_{(2, 27)} = 5.2$, $p = 0.0131$). No significant differences in otolith mean Sr:Ca content could be observed among areas (ANOVA, $F_{(2, 28)} = 1.0$, $p = 0.3859$).

Mean Sr:Ca of the 100 µm closest to the females' otolith's edge were positively correlated with surface water temperature (linear regression, $F_{(29)} = 5.8$, $p = 0.0227$; $r^2 = 0.17$, Figure 7.6 a) and surface water salinity (linear regression, $F_{(29)} = 4.9$, $p = 0.0345$; $r^2 = 0.15$, Figure 7.6 b). No linear relationships between environmental parameters and Ba:Ca as well as Zn:Ca could be observed.

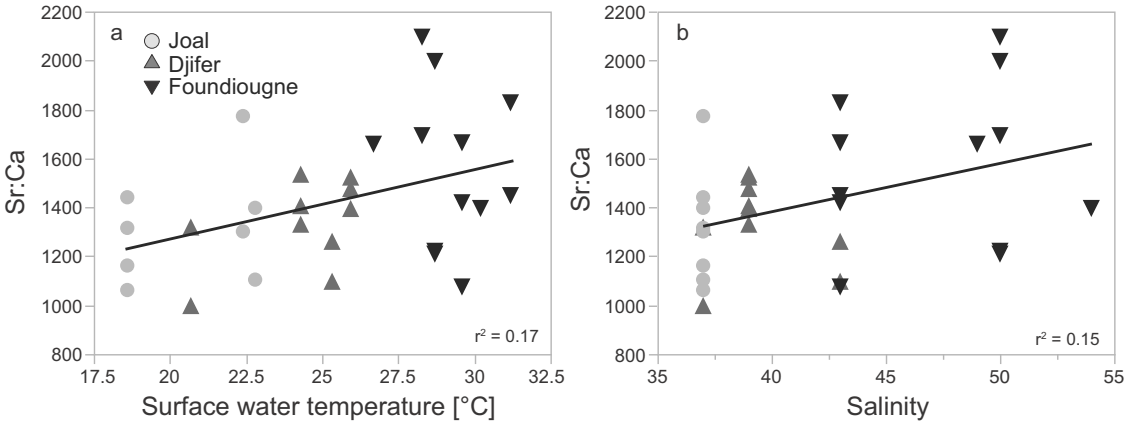

Figure 7.6 Relationships between mean Sr:Ca ratios (µg g-1) of female *Ethmalosa fimbriata* otoliths and water surface temperature (a) and salinity (b). Individuals were sampled at the Senegalese South Coast (Joal, light grey dots), at the Saloum River's mouth (Djifer, dark grey triangles), and inside the river's middle reaches (Foundiougne, black triangles)

All three elements are commonly used to discriminate between fish populations and were consequently employed for stock spawning component discrimination using LDFA. The LDFA plot (Figure 7.5 b) showed little separation between sampling sites. The cross-classification matrix (Table 2) revealed an intermediate percentage of correctly classified individuals (66.7, 60.0, and 81.8% for Joal, Djifer, and Foundiougne, respectively), with 27.3% individuals misclassified, while significantly different from random (Pillai's trace test $p = 0.0152$).

7.3.4. Identification of stock spawning components

The LDFA plot combining both classification techniques showed a clear separation between sampling sites (Figure 7.5 c). The cross-classification matrix integrating spawning energetics and otolith microchemistry yielded 95.4% correctly classified individuals (Table 7.1), while significantly different from random (Pillai's trace test, $p < 0.001$). Cross-validation revealed a mean of 10.0 ± 4.4% misclassified individuals with a mean -log2Likelihood of 15.0 ± 3.1.

Table 7.1 Cross-classification matrix of the linear discriminant analysis employing spawning energetics (oocyte dry weight, oocyte lipid and protein fractions, spawning batch energy content), otolith microchemistry (Ba:Ca, Sr:Ca, and Zn:Ca), as well as both techniques combined. **Bold** numbers display the percentages of correctly classified fish per sampling site. Sampling sizes are denoted by 'n'. Female *Ethmalosa fimbriata* were sampled at Joal (Senegalese South Coast), Djifer (Saloum River's mouth), and Foundiougne (Saloum middle reaches).

	Joal	Djifer	Foundiougne	n	Misclassified [%]	-log2Likelihood	p
	Spawning energetics						
Joal	**83.33**	0	16.67	6			
Djifer	40.00	**20.00**	40.00	5	36.36	38.0	0.1053
Foundiougne	9.09	18.18	**72.73**	11			
	Otolith microchemistry						
Joal	**66.7**	0	33.3	6			
Djifer	0	**60.0**	40.0	5	27.3	28.8	0.0152
Foundiougne	9.1	9.1	**81.8**	11			
	Spawning energetics + Otolith microchemistry						
Joal	**100**	0	0	6			
Djifer	0	**80**	20	5	4.6	6.5	0.0002
Foundiougne	0	0	**100**	11			

7.4. Discussion

Obtained results show that the combined use of spawning energetics and otolith microchemistry in the linear discriminant function analysis (LDFA) yields high classification percentages and thus illustrate distinct spawning stock components of Senegalese *Ethmalosa fimbriata*. A variety of reproductive life-history traits have been used to distinguish between stock spawning components, including timing, duration, and location of spawning; age, length, and weight at maturity; egg weight, size, viability and fecundity relationships; proportion of recruit and repeat spawners; and maternal effects as well as reproductive potential (Jakobsen et al. 2009). Also, assessments of the energy invested in reproduction are of ever-growing concern since this information is advantageous for stock component productivity appraisal and for bio-energetic model construction (Pecquerie et al. 2009). In recent years, the focus shifted increasingly towards elemental analysis of fish otoliths in order to discriminate among stock components (Kerr & Campana 2013).

E. fimbriata's Senegalese and Gambian stock is thought to be one population with an age-specific habitat use (Charles-Dominique & Albaret 2003). Juveniles are suspected to use estuaries as their main nursery ground (Gning et al. 2007), and large adults later on emmigrate to the sea (Charles-Dominique & Albaret 2003). This hypothesis, however, could not be supported by our observations on length frequencies distributions throughout the sampling sites at the Senegalese coast and within the Sine Saloum estuary. Spatial variations in spawner length distributions were negligible and may rather originate from differences in size-selectivity of gill nets used by local fishermen (Madsen et al. 1999).

Assessment of oocyte lipid and protein content allows for proper estimation of energy supply for embryonic development through the egg's yolk (Kamler 2005). Across all sampling sites (Joal: Senegalese South Coast, Djifer: Saloum River's mouth, Foungiougne: Saloum River's middle reaches), a linear correlation of oocyte lipid, as well as protein content with oocyte dry weight, was observed. The nutritional value of a given oocyte is, therefore, increasing with its size. In clupeids heavier/nutritionally rich oocytes are usually produced by females at the beginning of the spawning season, when water temperatures are comparably low and developmental times in early life stages are long due to metabolic constraints (Bradford & Stephenson 1992, Riveiro et al. 2004). *E. fimbriata* exhibits spawning in the hypersaline middle reaches of the Saloum River all year around (Panfili et al. 2004) and here the dry weights of the species' hydrated oocytes were shown to be significantly higher during the dry season than during the wet season (CHAPTER II). In the current study, mean energy contents of single hydrated oocytes of females sampled at Foundiougne were found to be significantly higher during the cold and dry season (March to May) than at the end of the wet season (October). This may consequently be a valuable adaptation to longer ontogenetic times due to lower water temperatures at the beginning of the year and to elevated

metabolic demands due to overall high salinities in the estuary's middle reaches during the dry season (Alderdice & Forrester 1968, Alderdice & Velsen 1971, Jatteau et al. 2017).

The energy invested into a single spawning batch (*SBEC*), however, remained temporally indifferent. Thus, *E. fimbriata* spawned more eggs but of lower energy content at Foundiougne during the wet season and vice versa during the dry season (CHAPTER I, CHAPTER II). Temporal trade-offs between fecundity and oocyte energy content seem to be a viable spawning tactic in clupeoid fishes to ensure early life stages survival (Castro et al. 2009, Döring et al. 2018a). Still, significant spatial differences in *SBEC* were encountered between individuals reproducing at the Senegalese South Coast and their counterparts spawning inside the Saloum River.

LDFA of spatially resolved spawning energetics yielded no significant result. The fact that Saloum River individuals invested a threefold more energy into reproduction, however, hints towards a generally higher productivity within the estuarine population. Apart from distinct spawning periods, former studies determined diverging values for the length at first maturity and differences in batch fecundity between the estuarine and the coastal population (Scheffers et al. 1972, Charles-Dominique & Albaret 2003, Panfili et al. 2004). Differences in these spawning traits are likely a result of variations in fishing pressure and adaptations to diverging hydrographic conditions (Jakobsen et al. 2009). In any case, varying individual reproductive investment ultimately alters a stock's productivity and consequently has been the basis of past stock spawning component discrimination in clupeids (Bradford & Stephenson 1992). Obtained results in the light of former studies, therefore, hint towards two productively distinct spawning components: one at the coast and one within the Saloum River.

The sampling sites at the Senegalese south coast and inside the Saloum River obviously have specific hydrographic dynamics. Annually reoccurring seasonal upwelling at the Senegalese South Coast affects water temperatures and thus chlorophyll levels/phytoplankton abundances (Ndoye et al. 2014, 2017, Tiedemann & Brehmer 2017). At the Saloum River's mouth, tides govern the exchange of water bodies, and inside the Saloum River's middle reaches high evaporation rates and little precipitation alter the hydrographic conditions throughout the year (Pagès & Citeau 1990). The water characteristics potentially print distinct signatures in the otoliths if females spent a significant amount of time (e.g. for spawning) within one of these habitats (Avigliano et al. 2017).

Water Sr concentrations are generally positively related to salinity in the Saloum River (8,000 µg l^{-1} at the mouth to 12,000 µg l^{-1} at Foundiougne) (Diouf et al. 2006). In the current study significant positive correlations of otolith

Sr:Ca levels with water temperatures as well as salinity could be observed. Highest Sr:Ca levels were recorded in otoliths of females sampled at Foundiougne, where water temperatures and salinities were always higher than at the other sampling sites (Pagès & Citeau 1990). Accordingly, it has been proposed before that at least water temperatures play an important role during incorporation of Sr since the process is associated with protein synthesis in relation to otolith crystallization rate. The formation rate of the proteinaceous matrix on the growing otolith surface is therefore thought to be correlated with somatic growth rates in marine fishes (Campana 1999). Somatic growth rates, however, are closely coupled to temperature and salinity in *E. fimbriata* (Panfili et al. 2004, CHAPTER I).

Environmental concentrations of Ba are generally higher in freshwater than in saltwater (Campana 1999), or transferable, in marine when compared to hypersaline waters. Correspondingly, otolith Ba:Ca levels were significantly higher in females sampled at the Senegalese south coast than in individuals caught inside the hypersaline estuary. Zn incorporation into the otoliths, on the other hand, is mainly influenced by fish diet and is supposed to be independent of water concentrations (Ranaldi & Gagnon 2008). Indeed, otolith mean Zn:Ca levels were significantly lower in individuals sampled inside the hypersaline estuary when compared to their counterparts sampled at the coast. Community composition of *E. fimbriata*'s main food item, phytoplankton, is subject to severe changes under hypersaline conditions (Blay Jr. & Eyeson 1982b, Nche-Fambo et al. 2015). Different species compositions may very well alter Zn incorporation into the female's otolith through differences in phytoplankton cell uptake rates and final concentrations (Sunda & Huntsman 1992). For this reason, Zn has been suggested as an indicator of habitat usage before (Ranaldi & Gagnon 2008). Especially in females sampled at Foundiougne high variances in otolith elemental:Ca values might be due to either migration of individuals or annually fluctuating temperature and salinity conditions (Pagès & Citeau 1990, Charles-Dominique & Albaret 2003). Nevertheless, significant differences in Ba:Ca and Zn:Ca give strong evidence of well-differentiated female habitat usage in the examined populations. The cross-classification matrix yielded percentages of correctly classified individuals which are marginally smaller than in a study conducted by Avigliano et al. (2017) on estuarine streaked prochilod *Prochilodus lineatus* (Valenciennes, 1837) using the same elements.

Marine fish stocks are comprised of several spawning components whose individuals are suspected to migrate between one another (Jakobsen et al. 2009). The LDFA integrating spawning energetics and otolith microchemistry yielded high classification success. Its application on *E. fimbriata* in Senegalese south coastal waters, therefore, resulted in the detection of spatially distinct spawning components. Our findings are in alignment with observations made in past studies (Charles-Dominique 1982, Charles-Dominique & Albaret 2003, Panfili et al. 2006, Durand et al. 2013) and the small geographical scale (15 – 45 km) on which population fractionation could be detected was comparable

to the one reported for American shad *Alosa sapidissima* (Wilson, 1811) (Melvin et al. 1992). The clear cut between spawning components at the coast, the Saloum River's mouth, and inside the river's middle reaches is most likely due to the estuary's distinct hydrological characteristics, which are markedly different from oceanic conditions. Heterogeneous hydrographic features lead to incorporation of varying element:Ca ratios into the female spawner's otolith and further require energetical adaptations to ensure recruitment success (Avigliano et al. 2017). As the low sampling sizes in the LDFA models suggest, assessing all investigated parameters for each individual fish is challenging. Standardizing both methodologies to analyze spawning energetics and otolith elemental fingerprint of clupeid fishes would improve the effort and simplify the execution. Given the high classification success of the final model, our approach of combining spawning energetics and otolith elemental fingerprints is a valuable method for stock spawning component identification in clupeid fishes.

7.5. Conclusion

Investigation of reproductive biology provides immediate insights into the processes responsible for the formation and maintenance of a fish species' stock structure. The basis of the biological definition of a stock is that they are reproductively isolated units, with individuals of each potential stock exhibiting homogeneous traits. Conversely, information on the stock structure is an essential asset to foster understanding of reproductive patterns. An individual female's ability to invest energy into reproduction is closely coupled with the stock's reproductive potential and thus with its productivity. We identified significant spatial differences in reproductive investment and otolith element:Ca profiles in Senegalese *Ethmalosa fimbriata*. This evidences several spawning components in southern Senegalese waters, which are characterized by delimited home ranges and distinctive productivity. Less productive components are more likely to become overexploited and a decline in catch rates could ultimately compromise the fisheries' activity and affect the depending socio-economic sector. Based on these findings, we suggest specialized ecosystemic management approaches for *E. fimbriata*'s stock, which comprises oceanic and estuarine spawning components.

7.6. Acknowledgements

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9. ADDITIONAL SCIENTIFIC CONTRIBUTIONS

9.1. Publications

Balde, SB, **Döring, J,** Ekau, W, Brehmer, P, (2018) Forecasts of population fecundity in *Ethmalosa fimbriata* (Bowdich, 1825) reveal spawning tactics in an upwelling environment. (*in prep*)

Tiedemann, M, Fock, HO, **Döring, J**, Badji, LB, Möllmann, C (2018). Water masses and oceanic eddy regulation of larval fish assemblages along the Cape Verde Frontal Zone. J Mar Syst. 183:42-55. https://doi.org/10.1016/j.jmarsys.2018.03.004

Döring, J, Neumann, SI, Sloterdijk, H, Ekau, W (2017) Seasonal growth differences of larval *Hyporhamphus picarti* (Hemiramphidae) in the Sine Saloum estuary, Senegal. J Appl Ichtyol. 34:97-102. https://doi.org/10.1111/jai.13528

Sloterdijk, H, Sadio, O, Brehmer, P, Müller, H, **Döring, J**, Ekau, W (2017) Composition and structure of the larval fish community related to environmental parameters in a tropical estuary impacted by climate change. Estuar Coast Shelf Sci. 197:10–26. https://doi.org/10.1016/j.ecss.2017.08.003

Tiedemann, M, Fock, HO, Brehmer, P, **Döring, J**, Möllmann, C (2017) Does upwelling intensity determine larval fish habitats in upwelling ecosystems? The case of Senegal and Mauritania. Fish Oceanogr. 26:655–667 https://doi.org/10.1111/fog.12224

Ndoye, S, Capet, X, Estrade, P, Sow, B, Machu, E, Brochier, T, **Döring, J**, Brehmer, P. (2017) Dynamics of a low-enrichment high-retention upwelling center over the southern Senegal shelf. Geophys Res Let, 44:5034-5043. https://doi.org/10.1002/2017GL072789

9.2. Datasets

Döring, J, Tiedemann, M, Stäbler, M, Sloterdijk, H, Ekau, W (2017): Reproductive parameters of *Ethmalosa fimbriata* as recorded in Senegal (Atlantic coast and Sine Saloum estuary). *PANGAEA*, https://doi.org/10.1594/PANGAEA.880051

Döring, J, Ekau, W (2017): Fatty acid compositions of *Ethmalosa fimbriata* oocytes sampled inside the Sine Saloum estuary (Senegal). *PANGAEA*, https://doi.org/10.1594/PANGAEA.880130

9.3. Theses co-supervision

Sarah Isabel Neumann (2016), "On the seasonal growth of the African halfbeak larvae (*Hyporhamphus picarti*) in the Sine Saloum estuary (Senegal)" Bachelor thesis (in German), University of Bremen, Leibniz Center for Tropical Marine Ecology (ZMT) Bremen, Germany.

Sebastian Kanne (2015), "Comparing the nutritional status of *Trachurus capensis* and *Engraulis encrasicolus* larvae under varying environmental conditions" Bachelor thesis (in German), University of Bremen, Leibniz Center for Tropical Marine Ecology (ZMT) Bremen, Germany.

Muhammed Oyinlola (2014), "The dynamics and reproduction potential of Bonga, *Ethmalosa fimbriata* (Bowdich, 1825, Clupeidae) in Cross River estuary and adjacent coastal waters" Master thesis, University of Bremen, Leibniz Center for Tropical Marine Ecology (ZMT) Bremen, Germany.

9.4. Teaching

Lecturer (2016), M.Sc. Program Marine Biology, Module E: Fisheries Biology, Systematics and Morphology of Fishes, University of Bremen, Leibniz Center for Tropical Marine Ecology (ZMT) Bremen, Germany.

Lecturer (2015), M.Sc. Program Marine Biology, Module E: Fisheries Biology, Systematics and Morphology of Fishes, University of Bremen, Leibniz Center for Tropical Marine Ecology (ZMT) Bremen, Germany.

9.5. Presentations

"Reproduction in *Ethmalosa fimbriata*: New insights into the species' spawning stock structure", **Julian Döring**, "Ecology of West African estuaries as important habitats for fisheries: a review and perspectives", workshop, Banjul, Gambia, 28 – 30 March, 2017

"Coping with hypersalinities: adaptations of *Ethmalosa fimbriata* to estuarine "inversification", **Julian Döring**, Hans Sloterdijk, Werner Ekau, 12th International Congress on the Biology of Fish, San Marcos, Texas, United States of America, June 12-16, 2016.

"Reproduction under hyperhaline conditions – batch fecundities and oocyte fatty acid compositions in *Ethmalosa fimbriata*", **Julian Döring**, Hans Sloterdijk, Werner Ekau, Symposium "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Bremen, Germany, February 29, 2016.

"Oocyte fatty acid compositions in *Ethmalosa fimbriata*", **Julian Döring**, Hans Sloterdijk, Werner Ekau, 2nd International Conference "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Dakar, Senegal, November 17-19, 2015.

"Drying out - "Inversification" of estuaries and the loss of their role as nursery area as a consequence of decreasing precipitation in West Africa with an example from Senegal, the Sine-Saloum Delta", Werner Ekau, **Julian Döring**, Hans Sloterdijk, Tropentag 2015, Berlin, Germany, September 16-18, 2015.

"Observations on the reproductive biology of *Ethmalosa fimbriata* (Bowdich, 1825) in Senegalese waters", **Julian Döring**, Ousseynou Samba, Werner Ekau, 1st International Conference "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Dakar, Senegal, December 9-11, 2014.

9.6. Posters

"Dynamics of a low enrichment-high retention upwelling center over the southern Senegal shelf", Siny Ndoye, Xavier Capet, Philippe Estrade, Bamol Sow, Eric Machu, Timothée Brochier, **Julian Döring**, Patrice Brehmer, PREFACE-PIRATA-CLIVAR Tropical Atlantic Variability Conference, Paris, France, November 28 – December 1, 2016

"Development and growth of *Hyporhamphus picarti* in an estuarine environment" Sarah I. Neumann, Hans Sloterdijk, **Julian Döring**, Werner Ekau, Mini-Symposium "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Bremen, Germany, February 29, 2016. https://doi.org/10.13140/RG.2.2.28037.65762

"Male clupeids increase spawning intensity in rapidly changing environments", Julian Döring, Hans Sloterdijk, Werner Ekau, 2nd International Conference "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Dakar, Senegal, November 17-19, 2015. https://doi.org/10.13140/RG.2.2.32630.50241

"Dynamical functioning of the southern Senegal upwelling as a new explanation of small pelagic spawning patterns", Siny NDoye, Xavier Capet, Philippe Estrade, Timothée Brochier, Eric Machu, **Julian Döring**, Patrice Brehmer, 2nd International Conference "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Dakar, Senegal, November 17-19, 2015.

"Distribution of pelagic fish eggs off the Senegalese Coast during an intense upwelling event in March 2014", **Julian Döring**, Timothée Brochier, Eric Machu, Xavier Capet, Oumar Manné, Werner Ekau, Patrice Brehmer, 1st International Conference "Ecosystem Approach to the management of fisheries and the marine environment in West African waters", Dakar, Senegal, December 9-11, 2014. https://doi.org/10.13140/RG.2.2.20886.45120

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Fine, thank you for supporting me in this venture. The weekends with you have been the best distraction from work I could have hoped for. With nothing but joy I am looking forward to our future 'joint venture'.

ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel

"Unravelling the reproductive tactics of a tropical clupeid fish (*Ethmalosa fimbriata*, Bowdich 1825) against the backdrop of climate change"

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkäre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

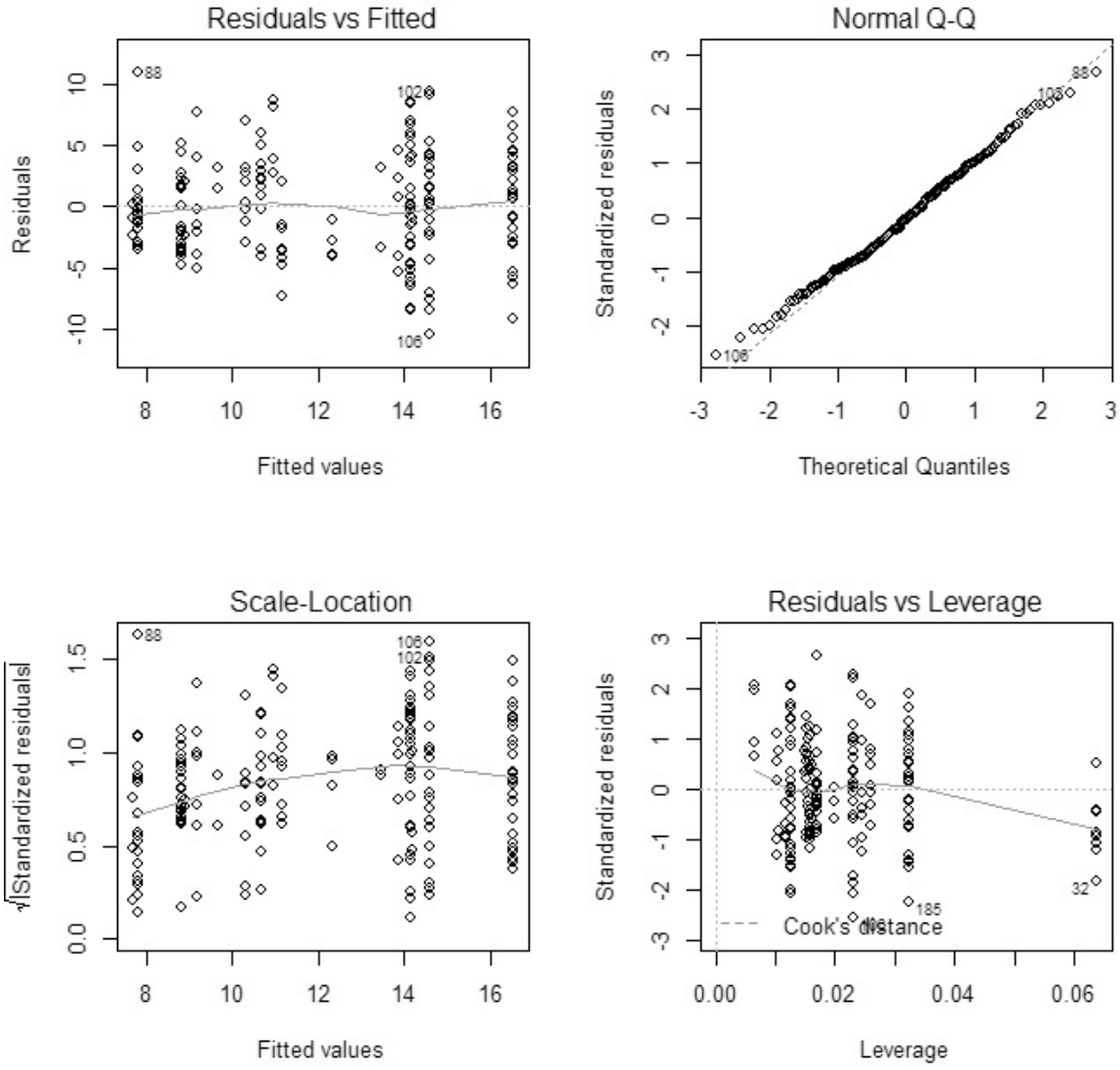
Bremen, 17.04.2018

_____

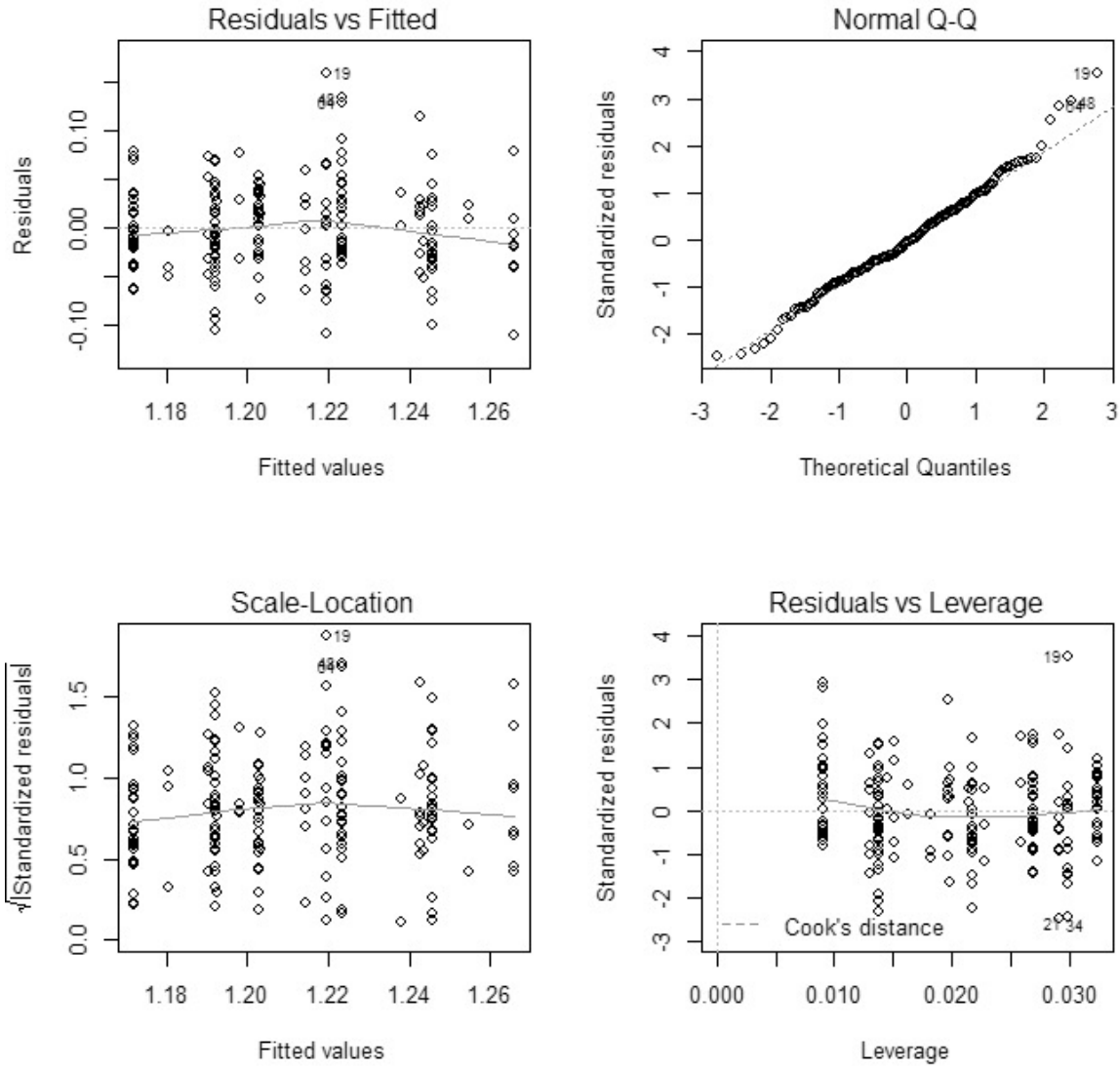
Julian Döring

APPENDIX

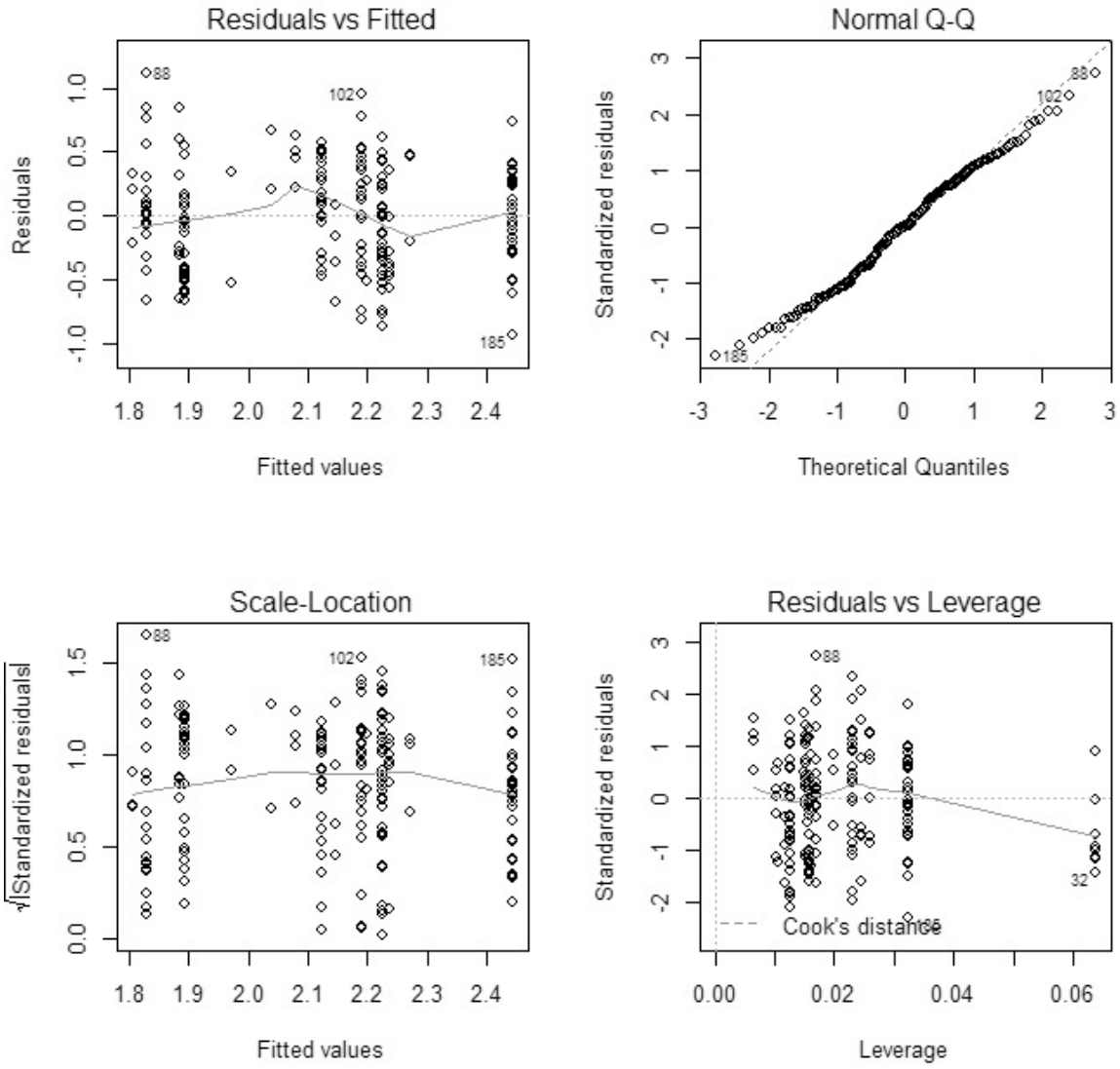
Appendix A



Appendix Figure 1 Residual plots for the multiple linear regression model fitted to the data on the square root of relative batch fecundity (*RBF*) against water surface temperature, and water surface salinity. Anderson-Darling tests and Cramer-von-Mises tests with a significance threshold of p = 0.1 did not show significant deviations of the residuals from a normal distribution



Appendix Figure 2 Residual plots for the multiple linear regression model fitted to the data on condition index (K_{hyd}) against water surface temperature, and water surface salinity. Anderson-Darling tests and Cramer-von-Mises tests with a significance threshold of p = 0.1 did not show significant deviations of the residuals from a normal distribution



Appendix Figure 3 Residual plots for the generalized linear model fitted to the data on gonado-somatic index (I_G) against water surface temperature, and water surface salinity. Anderson-Darling tests and Cramer-von-Mises tests with a significance threshold of p = 0.1 did not show significant deviations of the residuals from a normal distribution

Appendix Table A Range in total length (L_T), mean values for condition factor (K_{hyd}), gonado-somatic index (I_G), relative batch fecundity (*RBF*), and the range in absolute batch fecundity (*ABF*) of female *Ethmalosa fimbriata* by sampling site and month. Letters indicate Tukey HSD results, values not sharing the same letters are significantly different from each other ($p < 0.05$).

Site	L_T [cm]		February	March	April	May	June	July	August	September	October
Joal	23.1 - 27.5	K_{hyd}		1.56 (±0.12)	1.51 (±0.09)	1.51 (±0.10)					
		I_G		7.43 (±4.32)	6.97 (±2.05)	7.68 (±3.90)					
		RBF		72.21 (±144.48)	44.74 (±16.73)	79.33 (±76.024)					
		ABF		3,337 – 31,052	4,824 – 10,877	3,316 – 66,375					
Djifer	20.8 - 26.9	K_{hyd}	1.58 (±0.13)	1.61 (±0.03)	1.57 (±0.12)		1.57 (±0.12)	1.46 (±0.11)			
		I_G	10.32 (±3.08) ^{a}	7.20 (±4.19) ^{ab}	5.66 (±2.16) ^{b}		7.82 (±4.44) ^{ab}	12.70 (±2.08) ^{a}			
		RBF	145.05 (±76.78) ^{ab}	82.29 (±54.61) ^{bc}	64.53 (±50.97) ^{c}		99.68 (±97.95) ^{bc}	290.15 (±100.05) ^{a}			
		ABF	6,941 – 39,490	6,126 – 17,630	2,200 – 36,745		2,724 – 27,222	17,151 – 35,693			
Foundiougne	19.9 - 28.3	K_{hyd}	1.58 (±0.06) ^{a}	1.47 (±0.17) ^{a}	1.49 (±0.11) ^{ab}	1.45 (±0.05) ^{abc}	1.44 (±0.13) ^{abc}	1.47 (±0.07) ^{a}	1.39 (±0.11) ^{bc}	1.32 (±0.06) ^{c}	1.37 (±0.08) ^{c}
		I_G	12.17 (±3.88) ^{abc}	10.16 (±2.90) ^{abc}	6.73 (±2.11) ^{c}	8.61 (±4.55) ^{abc}	8.91 (±2.11) ^{abc}	10.92 (±4.84) ^{abc}	9.15 (±3.95) ^{bc}	13.00 (±4.36) ^{ab}	12.33 (±4.19) ^{a}
		RBF	144.36 (±27.62) ^{abc}	167.08 (±68.93) ^{bc}	90.36 (±26.89) ^{c}	189.96 (±123.84) ^{abc}	197.15 (±112.92) ^{abc}	247.87 (±150.02) ^{ab}	213.04 (±152.06) ^{bc}	226.78 (±96.53) ^{abc}	294.05 (±136.71) ^{a}
		ABF	16,388 – 23,160	6,470 – 54,205	6,344 – 13,298	13,173 – 29,889	11,229 – 36,199	3,160 – 85,409	4,321 – 85,045	19,501 – 37,985	6,162 – 61,270

Appendix B

Appendix Table B Results for the elemental analysis (µg g^{-1}) of the three different standards used for validation. NIES-22 was only measured once, measured mean values for BCR-2G and BHVO-2G are given including standard deviations.

Element	NIES-22		BCR-2G		BHVO-2G	
	Reference value	Measure value	Reference value	Measure value	Reference value	Measured value
Mn:Ca		0.12	*1550*	1500±65	*1317*	1364±62
Mg:Ca	*21*	18	*21468*	18963±3	*42996*	39771±4
Ba:Ca	*2.89*	3	*683*	667±16	*131*	133±3
Cu:Ca	*0.74*	1	*21*	17±0.3	*127*	124±3
Pb:Ca	*0.023*	0.031	*11*	10±0.4	*2*	1.7±0.1
Sr:Ca	*2360*	2308	*342*	339±5	*396*	405±8
Y:Ca		0.0089	*35*	33±1	*26*	25±0.4
Zn:Ca	*0.47*	1	*125*	146±3	*102*	113±2