

Influence of the physiological condition of bivalve recruits on their post-settlement dispersal potential

Martin Forêt^{1,2,*}, Frédéric Olivier^{2,3}, Philippe Miner⁴, Gesche Winkler¹,
Jean-Bruno Nadalini¹, Réjean Tremblay¹

¹Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

²Unité Mixte de Recherche 'Biologie des organismes et écosystèmes aquatiques' (BOREA, UMR 7208), Sorbonne Universités, Muséum national d'Histoire naturelle, Université Pierre et Marie Curie, Université de Caen Basse-Normandie, Université des Antilles, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement-207; CP53, 61 rue Buffon 75005 Paris, France

³MNHN, Station Marine de Concarneau, place de la croix, BP 225, 29182 Concarneau cedex, France

⁴Ifremer, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer, Centre de Bretagne, CS 10070, 29280 Plouzané, France

ABSTRACT: Secondary dispersal (= migration) of bivalves occurs after metamorphosis and is a key recruitment process that can radically change patterns of primary settlement. An example of secondary dispersal is active migration behavior of bivalve recruits such as in bysso-pelagic drift. We hypothesize that these active migrations represent an energy cost for recruits and that the ability to actively migrate will depend upon the recruit's physiological profile (quantity and quality of energy reserves). In lab experiments, we hatched 4 batches of recruits of *Venus verrucosa* with different physiological profiles by varying rearing temperature and diet composition. We then introduced these recruits into a fall velocity tube (5 m height) to estimate their vertical fall velocity as a proxy of their dispersal potential: slower fall velocity implies enhanced dispersal potential. We also compared alive vs. passive (dead) recruits to assess behavioral differences. Fall velocity increased logarithmically with recruit volume for each treatment, and no differences between active and passive individuals were observed for batches reared at 20°C with a mixture of *Tisochrysis lutea* and *Chaetoceros gracilis*. By contrast, active recruits in 2 other treatments (*T. lutea* at 20°C and a mix of *C. gracilis* and *T. lutea* at 15°C) significantly decreased their fall velocity regardless of their volume. Moreover, the ability of recruits to control their fall velocity by their behavior was correlated with triglyceride content. Recruits with the highest energy reserves had the greatest capacity to decrease their fall velocity, which suggests a major role of physiological conditions on potential secondary dispersal. We also used a benthic flume to test the substrate selection ability of recruits depending on their physiological profile and found no differences between physiologically different batches. However, *V. verrucosa* recruits preferred fine sediments, unlike adults, which live mainly in coarse sediment habitats; such difference in substrate preference suggests potential secondary migrations between nursery and adult areas.

KEY WORDS: Secondary migrations · Physiology · Behavior · Energetic reserves · Bivalve recruits · *Venus verrucosa*

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1. INTRODUCTION

In temperate marine systems, a large majority of benthic marine invertebrate species, including bivalves, exhibit a benthoplanktonic life cycle with a

pelagic phase preceding a benthic adult life (Thorson 1950). For all species, the renewal of populations is strongly linked to the success of recruitment, which will itself rely on several pre- and post-settlement processes (Pineda 2000). The importance of recruit-

ment on population structure of benthic invertebrates is strongly related to higher susceptibility of larvae and newly settled juveniles to stressful conditions and predation (Hunt & Mullineaux 2002, Qiu et al. 2002, Rayssac et al. 2010, Jenewein & Gosselin 2013). Recruitment can be influenced by several biotic factors, such as food availability and biological interactions such as predation, but also on abiotic factors related, for example, to hydrodynamics or substrate characteristics (Hunt & Scheibling 1997, Olivier & Retière 1998, Pineda et al. 2009). Post-settlement processes include migration, also known as secondary migration, as opposed to the pelagic primary larval dispersal phase.

When migrations are totally passive and related to hydrosedimentary dynamics, the dispersal of recruits is uncontrolled and also undesirable in cases of high sediment erosion (Emerson & Grant 1991, Armonies & Hellwig-Armonies 1992). By contrast, active migrations are triggered by a behavioral change, which for bivalves mainly involves byssus filament secretion. These filaments increase recruit buoyancy and drag and induce resuspension in the water column and subsequent bysso-pelagic drifting even at low shear stress velocities (Sigurdsson et al. 1976, Beukema & de Vlas 1989, Lundquist et al. 2004).

Although the role of hydrodynamic stress within the benthic boundary layer on post-settlement dispersal is now better understood, few studies have focused on biotic factors involved in active secondary migrations. However, recent studies have highlighted the crucial role played by the trophic environment in the recruitment process of benthic marine invertebrates, particularly the performance of larvae (growth and survival), larval release timing, and triggers for settlement (González-Araya et al. 2012, Toupoint et al. 2012, Kasten & Flores 2013, Jolivet et al. 2016). Moreover, Forêt et al. (2018a) showed that for several bivalves, recruit turnover, a proxy for secondary migrations (Armonies 1994b), was correlated with nanoeukaryote abundance in the water column, and they proposed the new concept of the 'trophic migration trigger.' Such synchronization would constitute a strategy to bear the energetic costs of bysso-pelagic drifting. However, given that growth performance of young recruits relies, at least in part, on food availability and quality, Forêt et al. (2018b) conducted a laboratory study to assess the potential duration of the temporal secondary migration windows for different species. They suggested that trophic dynamics and also temperature have a critical influence on spatial scales of secondary migrations by modulating bivalve growth rates, which are

also dependent on the physiological state of the bivalves; the faster a recruit reaches its resuspension size threshold, the lower is its secondary migration potential.

The aim of the present study was to assess the potential influence of bivalve recruit physiology on secondary migrations of a dominant species in the Chausey Archipelago (Normandy, France), the warty venus *Venus verrucosa*. We tested the hypothesis that the physiological status of newly metamorphosed juveniles, in terms of energy reserves and tissue essential fatty acid composition, supports active secondary migration behavior. Webb & Chu (1983) reviewed the role of biochemical components supporting bivalve larval development and concluded that lipids were the most important, particularly some polyunsaturated fatty acids identified as essential for larval growth and survival (see review by Glencross 2009). In order to test the influence of such biochemical parameters on secondary migration potential, we experimentally produced different batches of recruits with contrasting total fatty acid contents and essential fatty acid profiles by varying diets and temperature conditions during larval and post-larval development. The resulting 4 batches of recruits with different lipid characteristics were then used for 2 sets of experiments involving either a fall velocity tube or a benthic flume. We were thus able to (1) assess the ability of recruits to influence their dispersal potential by their behavior as a function of their physiological status (fall velocity measurements) and (2) study their potential for sediment habitat selection in relation to their physiological status during post-settlement dispersal (flume experiments).

2. MATERIALS AND METHODS

2.1. Larval and post-larval rearing

Larval and post-larval rearing was carried out at the Ifremer experimental hatchery (Plouzané, France). About 60 adult *Venus verrucosa* were collected in the Chausey Archipelago (48° 52' 50.21" N, 1° 49' 57.06" W) in July 2015 and were used for spawning induced by thermal shock and food addition (Gruffydd & Beaumont 1970). The different experimental conditions described below were applied 2 d post fertilization (dpf) when D-stage larvae were collected on a 45 µm square mesh screen. Larvae were reared in 150 l cylindro-conical tanks at an initial density of 5 larvae ml⁻¹ with filtered (1 µm) and UV-treated seawater maintained at constant temper-

ature (15 or 20°C, depending on the treatment group). Water was renewed, tanks were cleaned every 2 d, and stunted larvae were screened to eliminate moribund individuals to limit the mortality risk events related to potential bacterial developments. Larvae were fed once a day with *Chaetoceros gracilis* or *Tisochrysis lutea* or a mix of both species. The daily ration was equivalent to 40 cells μl^{-1} of *T. lutea* (equivalent cell volume) or a dry weight of $\sim 1 \text{ ng } \mu\text{l}^{-1}$. The volumes of *T. lutea* and *C. gracilis* were 42 and 73 μm^3 , respectively (Robert et al. 2004), and the ration was adjusted to take into account this difference.

After 21 or 27 dpf, depending on the rearing treatment, >50% of the population had developed into competent larvae (evidenced by the appearance of eyespot and foot). These larvae were transferred onto several downweller units (2 m \times 0.5 m \times 0.12 m with a 100 μm sieve) for metamorphosis and post-larval rearing. After this step, settlement was completed. Density was about 500 000 individuals per downweller, which was placed in a semi-open system with partial renewal of recirculated water, coupled with continuous food intake via a peristaltic pump from an aerated reserve tank that was filled every 2 d with seawater and algae. Food intake was increased during post-larval rearing to 100 cells $\mu\text{l}^{-1} \text{ d}^{-1}$ of *T. lutea* equivalent. Sieve size was increased steadily during rearing until a sieve size of 200 μm was reached. Microalgae were produced at 18°C in filtered seawater enriched with Conway medium and silicate (40 mg l^{-1}) for diatoms and maintained under continuous illumination from cool-white fluorescence lights at an intensity of 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and mixed with aeration (air + CO_2 : CO_2 input was regulated by pH control maintained at values between 7.2

and 7.8). *T. lutea* was produced continuously, whereas *C. gracilis* was produced in batches.

2.2. Experimental design

To produce batches of recruits with contrasting physiological states, we manipulated diet and temperature rearing conditions (Fig. 1). Three different diets were used at 20°C: 2 monospecific diet regimes with either *T. lutea* (T) or *C. gracilis* (C) (T20 or C20, respectively) and a mix of the 2 microalgal species (TC20). The same mix was used in the 4th treatment, but at 15°C (TC15). The 4 different treatments were triplicated for a total of 12 tanks.

Three successive developmental stages can be distinguished: pediveliger (before metamorphosis), post-larval (just after metamorphosis) and juvenile (at the end of the experiment at 60 dpf). After post-larval rearing, juveniles were transferred to the CRESCO/MNHN Marine Station (Dinard, France) for the fall velocity tube and benthic flume experiments. As seawater temperature could not be controlled in the experimental hall, the different batches were progressively reduced or increased to 18°C (room temperature), depending on the rearing conditions (15 or 20°C), during the last 5 d of post larval rearing (55 to 60 dpf).

2.3. Biochemical analysis

Lipid analyses were performed to quantitatively and qualitatively characterize the physiological status of the individuals reared under different conditions.

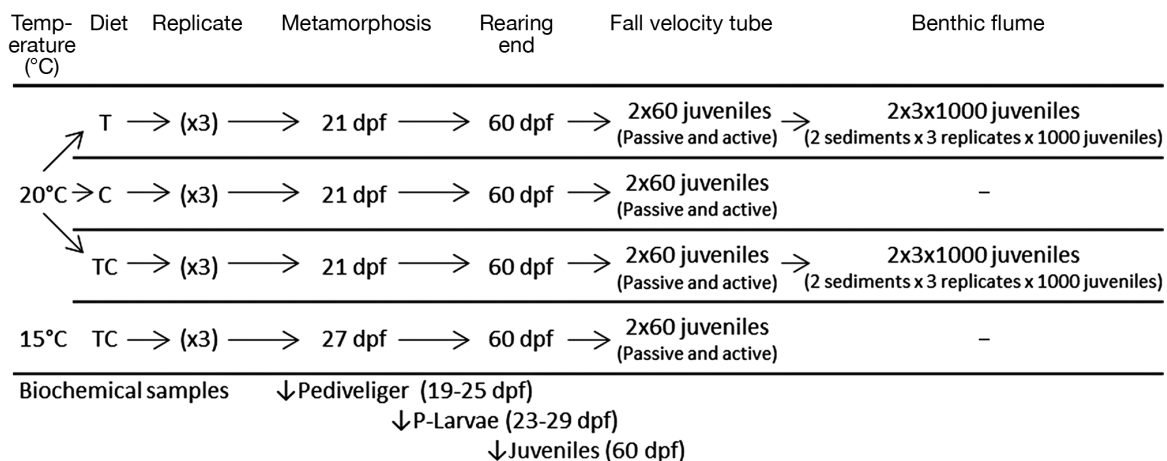


Fig. 1. Experimental design: 3 diets were tested, i.e. 2 monospecific regimens composed of *Tisochrysis lutea* (T) or *Chaetoceros gracilis* (C) and a mix of these 2 microalgae (TC) at different temperatures. Dates of sampling or changes in breeding conditions are expressed in days post fertilization (dpf)

Larvae were sampled before metamorphosis (19 or 25 dpf), post-larvae after metamorphosis (23 or 29 dpf) and juveniles at 60 dpf (just before pooling replicates and temperature acclimation for dispersal measurements; see Sections 2.4 and 2.5) for lipid analyses. Lipids were extracted following the procedure of Folch et al. (1957) using dichloromethane–methanol as modified by Parrish (1987) and separated into neutral (including triglycerides, free fatty acids and sterols) and polar (including mainly phospholipids) fractions by the methods developed by Marty et al. (1992). Briefly, silica gel hydrated with 6% water was used and eluted with 10 ml of chloroform:methanol (98:2 v/v) for neutral lipids followed by 20 ml of methanol for polar lipids. All fatty acid methyl esters (FAMES) were prepared as described by Lepage & Roy (1984) and analyzed in MSMS scan mode on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan), as described by Gendron et al. (2013). All FAMES were identified by comparison of retention times with known standards (37-component FAME Mix, PUFA-3, BAME and menhaden oil; Supelco) and quantified with tricosanoic acid (23:0) as internal standards. Chromatograms were analyzed using integration Xcalibur version 1.3 software (Thermo Scientific).

2.4. Fall velocity tube

The experimental equipment was detailed by Forêt et al. (2018b); briefly, a Plexiglas tube (5 m in height, 0.5 m in diameter) was used to measure fall velocities of bivalve recruits from the 4 rearing conditions. For each trial, a single juvenile, previously measured (length, width, thickness), was introduced at the top of the tube in 18°C seawater. Once the acceleration section passed (1.72 m), time needed to cover 2 m was measured (precision 0.1 s) and fall velocity was calculated. Juvenile behavior during the fall velocity measurements was noted (opening of the valves, foot and/or siphons protruding, etc.). Individuals were either introduced alive (active individuals) or dead after being immersed for 2 min in 4% formalin (passive individuals) in order to assess the impact of behavior on fall velocity. This method does not change the fall velocity value of fixed/killed compared to living individuals falling passively (Buchanan & Babcock 1997, Lundquist et al. 2004,

Olivier & Retière 2006, Petuha et al. 2006). These studies have shown that a low formalin fixation time (less than 5 min) allows killing juveniles without causing the valves to open and shell shape to change. About 60 active and 60 passive individuals were used for each rearing condition (total of 489 trials). As juvenile size at 60 dpf varied between each condition, we selected individuals between 431 and 1244 μm long to standardize and maximize the size range tested.

2.5. Benthic flume

The HYCOBENTHOS flume (Fig. 2) was used to estimate the habitat selection abilities of recruits for sediment preference under benthic boundary layer flows (Olivier et al. 1996a, De Montaudouin et al. 2003, Olivier & Retière 2006, Gagné et al. 2012). This racetrack benthic flume (10 \times 2.45 m) is 23 m long and 0.5 m wide. Flow is generated by the friction of 14 rotating disks immersed in the water and connected to a continuous electric engine. This set-up was primarily chosen to avoid any damage to small organisms when passing through the flow-generating system as may occur with pumps. An experimental section of 1 m length located opposite the flow-generating system was filled with 5 rectangular trays (1 m long \times 0.1 m wide \times 0.05 m deep). The central tray was considered the source zone where recruits were allowed to burrow at the beginning of each experiment. The other 4 trays were considered target zones that could only be colonized by migrating re-

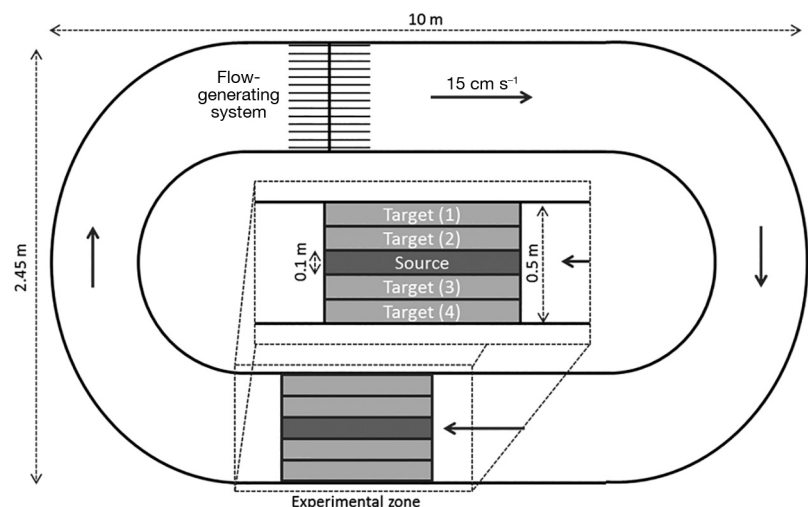


Fig. 2. Benthic flume (HYCOBENTHOS), highlighting the experimental section. *Venus verrucosa* recruits were deposited in the source zone located at the center of the experimental section. The sediment type (coarse or fine) in the target areas was randomly distributed among 2 zones for each type

cruits (see details in Olivier et al. 1996b and Fig. 2). One fine (<1 mm) and one coarse sediment treatment (>2 mm) were tested in triplicate in the source zone (total of 6 experiments). The different sediments used were collected from the preferred habitat of warty venus adults in the Chausey Archipelago and were sieved to obtain 2 fractions (<1 and >2 mm) and then defaunated by air drying.

For each experiment, the central source tray was filled with the sediment to be tested while the lateral target trays were randomly filled with either the fine or the coarse sediment treatment (2 zones for each type). Once the flume filled with filtered seawater at 18°C, juveniles were gently introduced at the surface of the sediment of the central tray with a pipette and then left for 7 h with no flow to limit potential stress effects after handling and to allow them to burrow into the substratum. Juveniles were then subjected for 14.5 hr to a unidirectional turbulent flow ($U_{\text{free stream}} = 15 \text{ cm s}^{-1}$; $u^* = 0.544 \text{ cm s}^{-1}$) under natural light to allow only active resuspension and avoid sediment erosion and thus passive emigration. At the end of the experiment, the flow-generating system was stopped, the flume was gently emptied, and the surface sediment in each tray was recovered and fixed with 3.5% formaldehyde. This material was then directly sorted by the elutriation ludox methods of Burgess (2001), modified by Toupoint et al. (2016), in order to collect bivalves from the sediment under a dissecting microscope.

To assess the role of physiological state on sediment habitat selection, we used 1000 juveniles from either the TC20 or T20 rearing conditions which were labeled and mixed before being introduced into the source zone. We used calcein labeling alternatively on 1 of the 2 batches according to methods detailed by Thébault et al. (2006) 72 h before the experiments. Briefly, juveniles were immersed for 4 h in a solution of 150 mg l^{-1} of calcein, then introduced in the flume. At the end of the experiment, sorted juveniles were observed under a fluorescence microscope (Olympus BX51) equipped with the FITC filter (emission/excitation wavelength 495/515 nm) to distinguish calcein-labeled vs. non-labeled individuals. The time between the labeling and the experiment as well as the labeling technique used limits the impact of the labeling on the behavior of the juvenile.

2.6. Statistical analyses

We used ANOVAs after assumptions of homoscedasticity and normality had been verified by Levene's

and Anderson-Darling tests, respectively. Differences in total lipid and triacylglycerol (TAG) concentrations were tested using a 1-way ANOVA followed by Tukey's post hoc test. Fatty acid relative abundances from 3 bivalve stages (pediveliger larva, post-larva and juvenile) and 4 treatments were used to create a matrix based on Bray-Curtis similarity distances used to produce non-metric multidimensional scaling (nMDS) representations. A 2-way PERMANOVA was then performed to test the effect of development stage, rearing conditions and their interaction on fatty acid profiles.

Analyses of covariance (ANCOVAs) were performed to compare passive and active fall velocity of each condition depending on juvenile volume. Individual juvenile volume was used as the covariate. Data of fall velocities and volume were linearized by inverse transformation ($1/x$). We first tested each of the fall velocity regressions as a function of volume and then compared the regressions of the 'live' to the 'dead' batches taking into account interactions, i.e. are regression slopes equal? When regression slopes were statistically equal, differences in intercepts between passive and active regression of the 4 conditions were used to calculate the ' Δ passive/active' parameter, which reflects the ability of juveniles in each condition to influence their fall velocity with their behavior. A regression was performed to investigate the relationships between Δ passive/active and TAG concentration. Differences in intercepts between regressions of passive and active groups (Δ passive/active) make it possible to quantify the capacity of juveniles of each condition to influence their fall velocity. This parameter was calculated from the transformed data and is dimensionless. The higher the value, the more juveniles are able to influence their fall velocity and thus their post-settlement dispersal potential. A 2-way ANOVA was used on retention rate obtained in benthic flume experiments, with rearing condition (T20 and TC20) and sediment type (coarse and fine) as factors. All statistical analyses were carried out with the software Minitab 17 except nMDS plots and PERMANOVA, which were realized with PRIMER v6 software.

3. RESULTS

3.1. Biochemical composition

Juveniles at 60 dpf showed significant differences in total lipid accumulation ($F_{3,11} = 7.25$ $p = 0.011$), with 2 to 3 times less lipids in TC20 compared to the

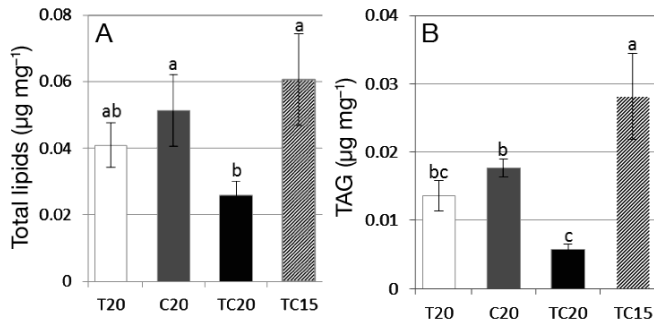


Fig. 3. (A) Total lipid concentrations and (B) triacylglycerol (TAG) concentrations of juvenile *Venus verrucosa* in each treatment (see Fig. 1 for treatment details). Data are mean \pm SD (n = 3). Different letters indicate significant differences (Tukey's test)

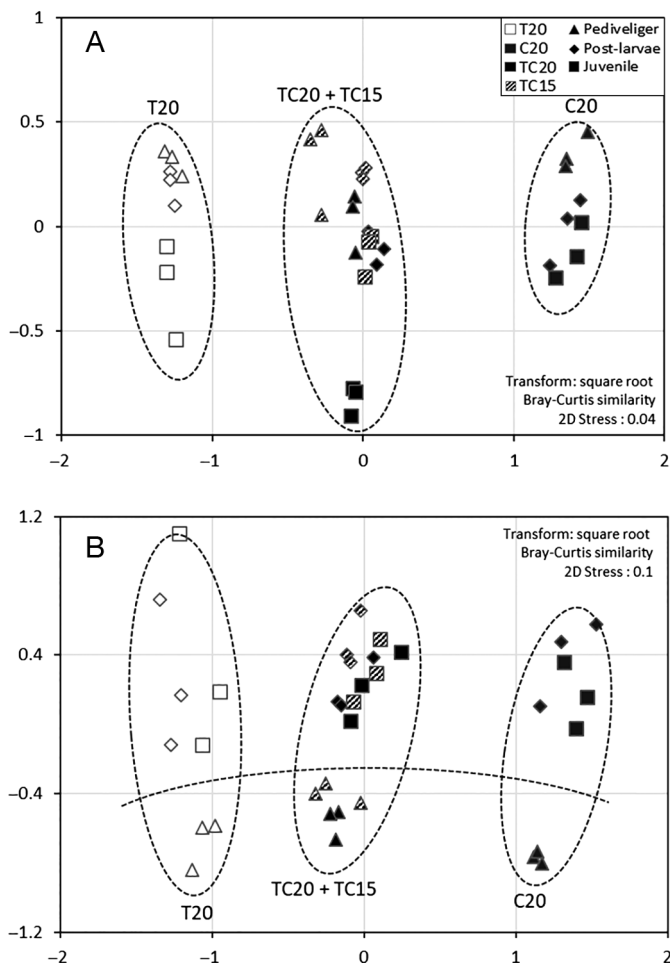


Fig. 4. Non-metric multidimensional scaling on (A) neutral and (B) polar fatty acid profiles for 4 rearing conditions and 3 developmental stages of *Venus verrucosa*. Data were square root transformed, and the similarity matrix is based on the Bray-Curtis index. Dashed ellipses distinguish the 3 different diets (T, C and TC; see Fig. 1 for treatment details), and the dashed curve indicates the effect of the metamorphosis of polar fatty acid profiles as determined by PERMANOVA

other treatments (Fig. 3A). These results were similar to TAG content ($F_{3,11} = 22.43$, $p < 0.001$), with the lowest values measured in juveniles reared in TC20 and the highest in TC15 (Fig. 3B).

The nMDS representations of dissimilarities in fatty acid profiles between different stages (pediveliger larva, post-larva and juvenile) and rearing treatments are shown according to either neutral (Fig. 4A) or polar lipid fractions (Fig. 4B). In the neutral fraction, the effect of the diet is clearly visible on fatty acid profiles of juveniles and distinguishes each of the 3 diets regardless of rearing temperature, with mixed diets (TC20 and TC15) showing a mixture of the 2 mono-specific regimes (T20 and C20). Similar results were obtained in the polar fraction of juveniles in addition to the distinctness of the pediveliger from post-larval and juvenile stages, suggesting a high metamorphosis effect on polar fatty acid profiles. Results of PERMANOVAs showed a significant interaction effect among stages and rearing conditions on the neutral (pseudo- $F_{6,35} = 4.02$, pseudo- $p = 0.0003$) and polar fatty acid profiles (pseudo- $F_{6,35} = 1.74$, pseudo- $p = 0.0455$). However, the pairwise tests were not significant. The percentages of similarity between each condition at the juvenile stage (neutral and polar fraction) are shown in Table 1.

3.2. Fall velocity tube: dispersal potential

As fall velocity is mostly dependent on the volume of a particle, the relationship between shell length and juvenile volume is shown in Fig. A1 in the Appendix. Fall velocity as a function of juvenile volume for each condition is presented in Fig. 5. The regression tests were significant ($p < 0.001$) for each of the 8 regressions tested (4 treatment conditions and status either active or passive). There was no interaction be-

Table 1. Average similarity (%) of fatty acid profiles (neutral and polar fractions) among juvenile *Venus verrucosa* in each rearing condition. See Fig. 1 for details of rearing conditions and experimental design

		T20	C20	TC20	TC15
Neutral	T20	95.48			
	C20	70.52	95.50		
	TC20	83.29	80.00	98.52	
	TC15	84.02	84.28	90.58	96.96
Polar	T20	91.81			
	C20	77.14	94.34		
	TC20	86.57	84.83	93.35	
	TC15	86.66	85.30	93.05	94.67

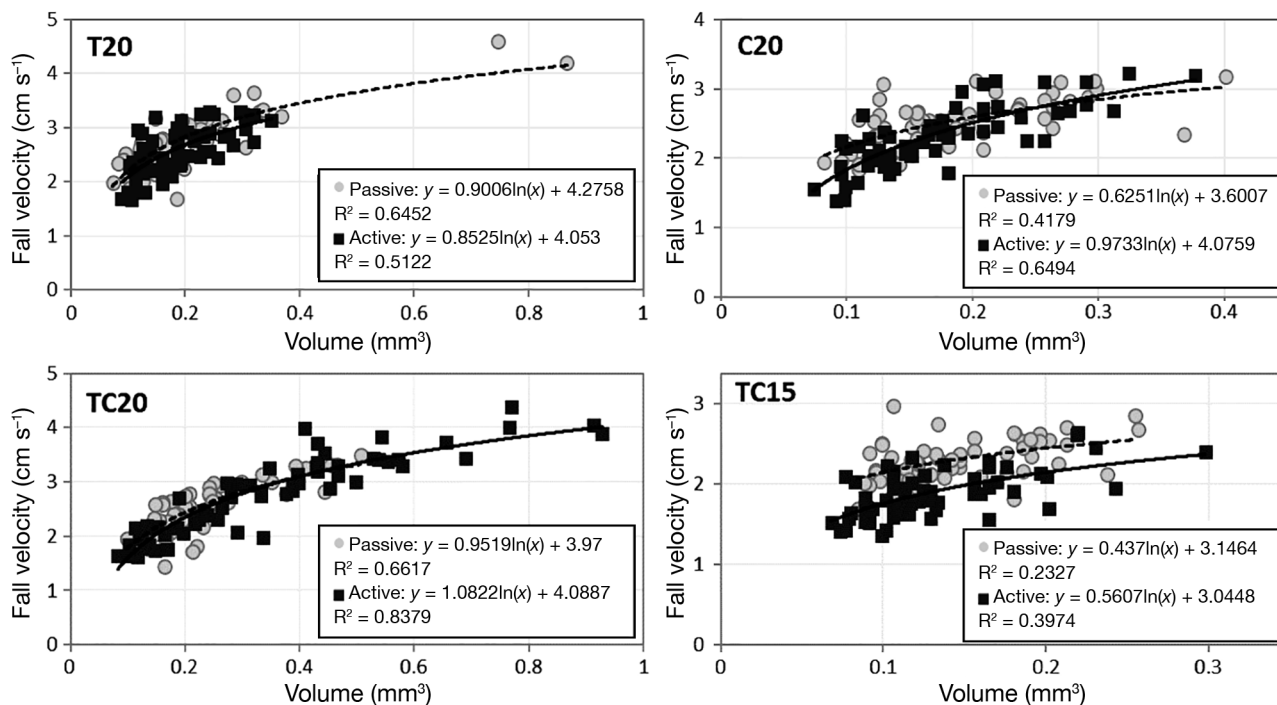


Fig. 5. Relationships between fall velocity and recruit volume for passive (gray symbols, dashed line) and active *Venus verrucosa* recruits (black symbols, solid line) in each treatment (see Fig. 1 for treatment details). For all regressions: $p < 0.001$

tween volume and status on fall velocities for batches T20, TC20 and TC15. ANCOVA showed that active individuals fell more slowly than passive individuals in T20 ($F_{2,121} = 6.36$, $p = 0.013$) and TC15 ($F_{2,121} = 24.03$, $p < 0.001$), i.e. in these 2 conditions, juveniles were able to influence their fall velocity with their behavior.

For C20, there was a significant interaction between volume and status ($F_{2,118} = 6.97$, $p = 0.009$); the slopes of the regressions were therefore not equal. Thus, for this treatment, the datasets of active and passive juveniles were split into 2 sub-groups (Fig. 6) of small and large individuals (volume lower or higher than the median of 0.16 mm³). New regression tests were then performed on transformed data (1/x). No interactions between volume and status were found for small or large individuals. Falling speeds were different between active and passive individuals only for small individuals ($F_{2,59} = 11.59$, $p < 0.001$), while no significant effect was observed for larger individuals ($F_{2,61} = 0.11$, $p = 0.744$). Only C20 individuals whose volume was < 0.16 mm³ (670 μm length) reduced their fall velocity with their behavior.

The size of juveniles introduced into the fall velocity tube ranged from 431 to 1244 μm or 0.049 to 0.928 mm³. The minimum and maximum measured fall velocities were 1.29 and 4.38 cm s⁻¹, respectively. Various active behaviors were observed during the fall, such as the opening of the valves or the protrusion of structures such as the foot or siphons. How-

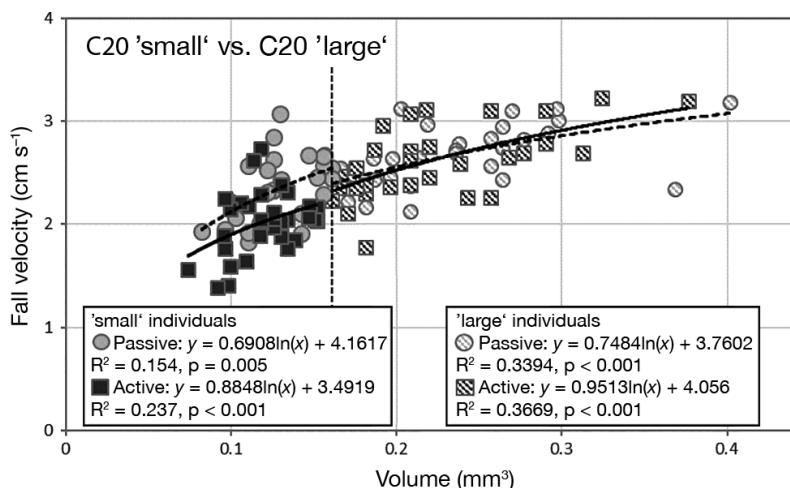


Fig. 6. Relationship between fall velocity and recruit volume for passive and active recruits of *Venus verrucosa* in 2 size classes ('small' and 'large') in the C20 treatment (see Fig. 1 for treatment details). Dashed and continuous curves correspond respectively to regressions of passive and active individuals, and the vertical dashed line corresponds to the median size in C20 (0.16 mm³)

ever, the small size of juveniles made it difficult to systematically observe their behavior, and therefore their ability to influence their speed of fall cannot be clearly linked to any particular behavior. Δ passive/active values were 0.024, 0.0574, 0.0075 and 0.0855 for T20, C20 (small individuals), TC20 (no significant differences between passive and active, as shown by ANCOVA) and TC15, respectively. The Δ passive/active parameter was significantly correlated with TAG concentration ($p < 0.001$, $R^2 = 0.859$; Fig. 7).

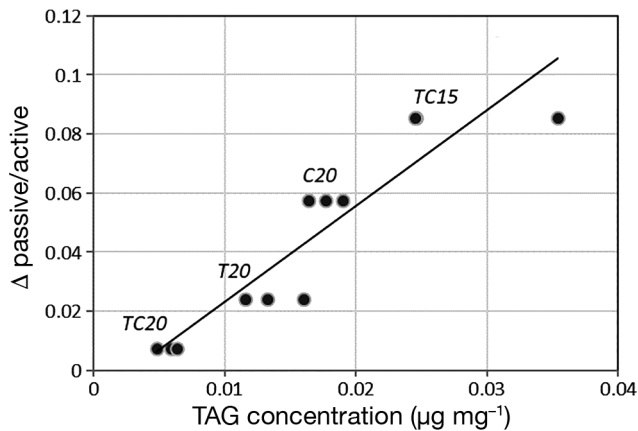


Fig. 7. Relationship between the Δ passive/active parameter, i.e. the difference in intercepts between regressions of passive and active juveniles, which reflects the ability of individuals in each condition to influence their fall velocity by their behavior, in all treatments as a function of triacylglycerol (TAG) concentration of each of the 3 replicates ($N = 12$, $p < 0.001$, $R^2 = 0.849$). Labels indicate rearing conditions (see Fig. 1 for details), and data from 2 replicates of the TC15 condition overlap at $\text{TAG} \approx 0.025 \mu\text{g mg}^{-1}$

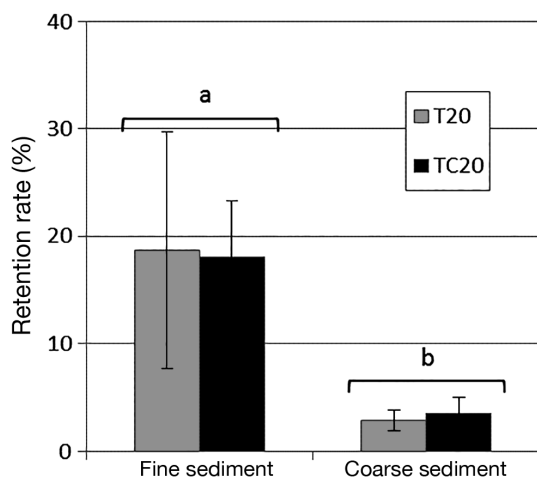


Fig. 8. Retention rate of *Venus verrucosa* juveniles in the T20 and TC20 treatments (see Fig. 1 for treatment details) according to the sediment type of the source zone in the benthic flume (see Fig. 2 for flume details). Recruits ($n = 1000$) from each treatment were used in each experiment, with 3 replicates per type of sediment ($p < 0.001$). Different letters indicate significant differences (Tukey's test)

3.3. Benthic flume: substrate selection ability

Juvenile retention rates did not significantly differ between T20 and TC20 ($F_{3,11} < 0.01$, $p = 0.988$). In contrast, the retention rate in the source zone was 6 times higher in fine than in coarse sediment treatments ($F_{3,11} = 20.41$, $p < 0.001$; Fig. 8). The colonization rate of target zones was negligible, with a maximum of 0.5% cumulative recolonization.

4. DISCUSSION

For the first time, this study shows that post-metamorphic migratory behavior (i.e. the ability to influence fall velocity and thus dispersal potential) can relate to the physiological status of young juveniles of an endobenthic bivalve species. The Δ passive/active parameter was considered here as a proxy of the ability of a recruit to modulate its fall velocity, and a strong significant correlation with neutral lipid (TAG) concentration was observed. Thus, our hypothesis that the physiological status of newly metamorphosed juveniles supports active secondary migration behaviors is supported. Juveniles accumulating more lipids would be more active, showing higher potential for secondary migration. However, fatty acid composition of juveniles was not related to their secondary migration potential.

Lipids are the main source of energy for marine organisms (Bergé & Barnathan 2005). The 2 main classes of lipids for bivalve larvae are phospholipids and triglycerides, with structural roles especially for the lipid membranes for phospholipids and energetic roles for triglycerides (Holland 1978, Gallager et al. 1986, Delaunay et al. 1992). The physiological state of bivalve larvae and post-larvae is essentially controlled by the abiotic and biotic conditions to which they are exposed (Pechenik 1990, Pernet et al. 2007a). Their lipid composition is therefore mainly influenced by their diet (Nevejan et al. 2003, Pernet et al. 2005, Gagné et al. 2010) but also by temperature (Pernet et al. 2007b, Rayssac et al. 2010, Barret et al. 2017). The influence of temperature on the accumulation of TAG varies according to species and their thermal preferences. Ben Kheder et al. (2010) observed, as we did, a greater accumulation of lipid reserves at low temperatures in *Crassostrea gigas*, whereas Barret et al. (2017) observed a greater accumulation of TAG at 20°C than at 12°C in American lobster *Homarus americanus*. TAG content strongly influences biological performances at both larval and post-larval stages, positively correlated to survival

and growth rates, but also to more efficient habitat exploration and selectivity during settlement (Thiyagarajan et al. 2002, Nevejan et al. 2003, Pernet et al. 2003, 2004, Tremblay et al. 2007). TAG concentration is thus considered an excellent indicator of the physiological quality of bivalves (Fraser et al. 1989).

Furthermore, our work shows for the first time the influence of the physiological condition on active drifting of young bivalve juveniles. Pernet et al. (2003) already showed a link between recruitment mechanisms and physiology but for other stages of development and recruitment mechanisms: settlement behavior of larvae for habitat selection (primary settlement) was directly linked to the larval TAG content. They demonstrated that individuals with higher accumulated energy content delayed their metamorphosis, which could allow them to extend the phase of habitat/substratum selection and maximize post-settlement survival. Moreover, whereas TAG content is a proxy for the physiological status of bivalve juveniles, it does not correspond to a direct measurement of the metabolic activity of such individuals and thus the direct link between energy reserves and migration behavior remains to be confirmed.

We also observed for one monospecific diet at 20°C that smaller individuals fell more slowly than larger individuals. Our set of experiments did not allow us to explain the reason for this difference, which may have been (1) because of different TAG levels in the 2 size classes or (2) because small and large mussel recruits with the same level of energy reserves cannot actively control their dispersal through their behavior once they exceed a certain size threshold. However, our observations show that the active dispersal potential of a recruit decreases with age, as already demonstrated for bysso-pelagic drift mechanisms (Sigurdsson et al. 1976, Beukema & de Vlas 1989). Indeed, mussel recruits would be able to produce byssus threads up to 2.5 mm. Possibly, depending on the accumulated reserves (TAG content), the energy needed by recruits to influence their fall velocity, and therefore their dispersal, would become too great regardless of the behavioral mechanism once they have passed a certain size/volume threshold. Such a threshold size/volume would therefore be related to both physiological state and to a size/volume passive effect.

Marshall et al. (2003) and Pechenik et al. (1998) demonstrated that trophic conditions during the larval phase could have delayed impacts on post-metamorphic rather than larval life. Such a 'latent effect' is particularly critical in the case of food deficiency (quantity and/or quality) at the larval stage by

decreasing biological performance after metamorphosis. In fact, the recruits' performances (survival, growth etc.) will therefore not only reflect post-metamorphic biotic and abiotic conditions, but also previous larval conditions. On the other hand, the 'desperate larva' hypothesis developed in the 1950s (Knight-Jones 1953, Wilson 1953) argues that during the competence phase, the larva consumes its energy reserves until it reaches a threshold value below which its selectivity for suitable settlement sites decreases until no choice can be made. This may thus lead to primary settlement on unsuitable habitats rather than risking death before metamorphosis (Botello & Krug 2006). Secondary migrations can allow post-metamorphic stages that have settled onto unsuitable sites (as in the case of the desperate larva hypothesis) to leave and reach a more favorable habitat for their development (Armonies 1994a, Olivier et al. 1996b). However, older larvae, which have delayed their metamorphosis and thus have less energy content, should exhibit a reduced ability to undergo post-settlement migrations.

In our fall velocity tube experiments, we were not able to systematically and clearly identify the specific behaviors of *Venus verrucosa* modulating the fall velocity, as the juveniles were too small for detailed direct observations. However, we suspect that valve opening and, to a lesser extent, foot/siphon protruding would be more common behaviors for decreasing the fall speed by increasing the shear drag in comparison to production of byssus filaments (bysso-pelagic drifting). The energy cost of bysso-pelagic drifting has never been estimated, but similar byssus filament production can represent 15% of the total energy budget of adults (Griffiths & King 1979, Hawkins & Bayne 1985) and even more under stress conditions (Cheung et al. 2006). Adults in poor energetic condition invest less energy in byssogenesis, with lower production of thread of a smaller diameter (Babarro et al. 2008, Hennebicq et al. 2013). We can thus assume that for the warty venus, energy costs related to bysso-pelagic drifting could be relatively high for young metamorphosed stages, leading them to favor valve opening as a more energy-efficient behavioral means of active secondary dispersal. The interpretation of the positive relationship between TAG content and active dispersal efficiency, involving such low energy consuming behavior, is thus difficult. However, the cost of dispersal is not limited to the metabolic component of migratory behavior, but also includes components such as time, risk and opportunity (Bonte et al. 2012). Temporal costs represent the time invested in dispersal that cannot be

dedicated to other functions, such as feeding. Risk costs are related to greater probabilities of mortality during dispersal (Hiddink et al. 2002), while opportunity costs result in hazards of reaching unsuitable habitats during secondary migrations. Within that context, juveniles which have not accumulated enough energy reserves to sustain all of these costs associated with post-settlement dispersal should not migrate. There is thus a trade-off between the fitness advantage to disperse after settlement and the costs and risks that are a function of the physiological state of the recruit and therefore of its life history. As an example, such a mechanism has been demonstrated for fish migrations in response to seasonal changes in predation and growth (Brönmark et al. 2008). Migration mechanisms are then the result of seasonally fluctuating trade-offs between predator avoidance and foraging gains.

While warty venus adults preferentially inhabit coarse soft-sediment habitats (Tebble 1966, Poppe & Goto 1993, Arneri et al. 1998), our flume results show increased juvenile retention rates in fine compared to coarse sand. Such a change in substrate preference between adults and recruits could suggest the existence of nursery areas associated with fine sand and secondary migrations once a refuge size is reached to colonize the sites of main adult populations as already demonstrated for *Macoma balthica* in the Wadden Sea (Beukema & de Vlas 1989).

Various factors may account for the existence of primary fixation zones, such as the nature of the substrate, with some species preferentially fixing on filamentous substrates (Eyster & Pechenik 1988, Dobretsov & Miron 2001). Selection of a primary settlement microsite could also be related to diet, as young bivalves are able to feed by using their foot (pedal feeding) on microphytobenthic biofilms (Gatenby et al. 1997, Beck & Neves 2003) or chemotaxis perception of cues often associated with bacterial biofilms (Hadfield 2011). Moreover, nursery areas could limit adult–recruit competition (Porri et al. 2008) that can lead to a decrease in settlement success due to cannibalism, competition for space or food and bioturbation activity of adults (André et al. 1993). Finally, several features restricted to fine sediment habitats should be sufficiently positive in the settlement/metamorphosis period to justify costs and risks of secondary migrations. Indeed, Forêt et al. (2018a) showed that whereas the migration potential of *V. verrucosa* recruits was relatively low compared to other bivalve species, such a disadvantage could be compensated by low post-larval growth rates that indirectly increase the temporal windows of migra-

tion. In addition, as suggested in our study, metabolically inexpensive migration behaviors (valve opening) would also limit the energy risk associated with dispersal.

To conclude, we produced 4 batches of warty venus juveniles with different physiological profiles by varying rearing temperature and diet composition. These 4 batches differed particularly in terms of TAG content and to a lesser extent in terms of fatty acid profiles. We used these individuals to test the influence of their physiological state on their ability to influence their post-settlement dispersal by their behavior and also on their potential for sediment selection during their first weeks of life after metamorphosis. The individuals with the highest energy contents were most likely to influence their fall speed and thus potentially increase their post-settlement dispersal. This confirms our hypothesis that the migration potential of bivalve juveniles is linked to their physiological status, more particularly by the amount of energy accumulated, and therefore by the environmental and trophic conditions encountered during the larval and post-larval stages. Although we did not observe any influence of physiology on substrate selection processes, we have nonetheless shown a clear preference of warty venus juveniles for fine sediment, whereas adults of this species showed a clear preference for coarse sediment habitats, thus suggesting significant secondary migration mechanisms.

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Appendix. Supplementary data

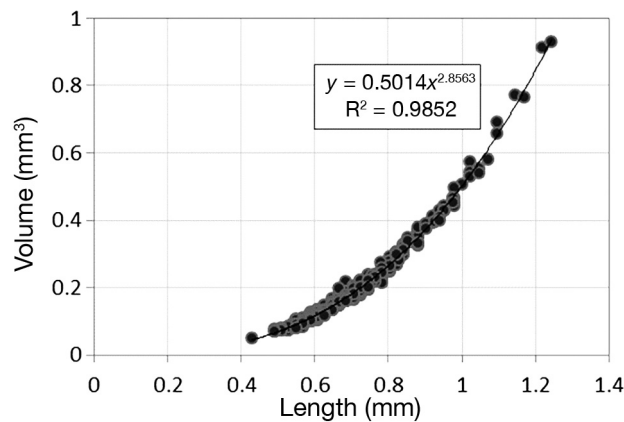


Fig. A1. Relationship between *Venus verrucosa* shell length and estimated volume of recruits (n = 489)