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Match/mismatch between the *Mytilus edulis* larval supply and seston quality: effect on recruitment

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Abstract:

We considered Cushing's match/mismatch theory in a heterotrophic environment and hypothesized that settlement and recruitment success in blue mussel are higher when the food supply is rich in polyunsaturated and essential fatty acids (PUFA/EFA). To test this hypothesis, we monitored larval development as well as fatty acid composition in trophic resources during two successive reproductive seasons. The decoupling we found between the presence of competent larvae in the water column and settlement rates strongly suggests that metamorphosis is delayed until conditions are suitable. In both years, the major mussel settlement peak was synchronized with a phytoplanktonic pulse rich in EFA, consisting of a large autotrophic bloom in 2007 and a short but substantial peak of picoeukaryotes in 2008. These results suggest a "trophic settlement trigger" that indirectly affects recruitment by strongly improving the settlement rate. Despite similar larval settlement rates during both years, the lower 2007 recruitment likely resulted from a mismatch with a high lipid-quality trophic resource. The seasonal trophic conditions differed greatly between the two years, with fatty acids profiles reflecting heterotrophic plankton production in 2007 and mostly autotrophic production in 2008. In agreement with Cushing's theory, our results highlight a match/mismatch, related to the food lipid quality rather than food quantity. For the first time, we show that the recruitment in marine bivalves may be dependent on phytoplanktonic pulses characterized by high levels of PUFA.

Keywords: fatty acids ; larval supply ; match/mismatch ; *Mytilus edulis* ; picoeukaryotes ; recruitment ; settlement rate ; trophic settlement trigger

Introduction

The blue mussel (*Mytilus edulis*, L. 1758) has a complex life history, with a dispersive planktotrophic larval phase lasting for several weeks until settlement and metamorphosis into benthic post-larvae. Mussel larvae are omnivorous (Raby et al. 1997), feeding mainly on the

seston's particulate organic matter (POM) that includes living organisms such as bacteria, cyanobacteria, phytoplankton (eukaryotes) and heterotrophic protists. The metamorphosis requires a high expenditure of energy at a time when individuals have limited feeding ability due to degenerated velum and undeveloped gill ciliature (Bayne 1971). Biochemical reserves accumulated during the larval phase (Videla et al. 1998) determine both metamorphic success (Pernet et al. 2004) and young juvenile performance (Phillips 2002, 2004).

Lipids constitute a major source of metabolic energy and an essential material for cell and tissue formation (Bergé and Barnathan 2005). Fatty acids are the main components of lipids and their composition reflects the physiological condition of an organism. Marine animal cells can synthesize *de novo* saturated and monounsaturated fatty acids (SFA and MUFA respectively), but biosynthesis of major polyunsaturated fatty acids (PUFA) is impossible or very limited due to the lack or limited activity of specific enzymes (see review of Glencross 2009). Consequently, trophic resource must provide bivalves with the needed PUFA, such as eicosapentanoic, arachidonic, and docosahexaenoic acids, which are commonly considered as essential fatty acids (EFA; Parrish 2008). The structural and metabolic roles of EFA ensure the optimal growth (Langdon and Waldock 1981, Delaunay et al. 1993), and the settlement and metamorphic success of bivalve larvae (Soudant et al. 1998, Pernet and Tremblay 2004).

Cushing's match/mismatch theory (1990) was developed to explain variations in herring recruitment success, and based on the synchronization (match) or the desynchronization (mismatch) between production cycles of larvae and their prey. This hypothesis was repeatedly updated and validated on marine invertebrates (Philippart et al. 2003, Bos et al. 2006, Ouellet et al. 2007), but classically related to the food abundance (e.g., Durant et al. 2005) and composition (e.g., Beaugrand et al. 2003). In this study, we propose that settlement and recruitment success in

blue mussel relates with high levels of dietary EFA. We consider settlement as the process by which individuals become associated with the substrate and recruitment as the number of individuals at time *t* of the benthic life.

Our hypothesis was tested in a coastal lagoon characterized by a trophic food web based mainly on heterotrophic production, which is slightly supplemented by autotrophic nanoplankton production, and very short and occasional diatoms blooms (Trottet et al. 2007). Despite the potential ability for *de novo* EFA synthesis in heterotrophic protists (e.g., Bec et al. 2010), this type of food is generally considered to be of poor dietary value for bivalves due to a low PUFA/EFA ratio (Sargent et al. 1999). With the aim of testing Cushing's match/mismatch theory with the food lipid quality, we monitored the larval development of mussels, and their settlement and recruitment dynamics in parallel with seston characteristics. We tested the hypotheses that the abundance of dietary EFA controlled both settlement (H1) and recruitment (H2) success.

Methods

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Sampling design

The study was carried out in a coastal lagoon (Bassin du Havre-Aubert, BHA) of the Îles de la Madeleine (Québec), an archipelago located in the Gulf of St. Lawrence (195 000 km²) in eastern Canada. The characteristics of BHA allowed us to easily track the production and recruitment of different cohorts of mussel larvae. Connected to the open sea by a narrow channel in the south (Fig. 1a), BHA is a restricted lagoon of only 3 km² (mean depth 2–3 m), which is submitted to a microtidal regime (mean tidal range ~ 0.3 m) and a strong wind influence. Local wild mussel populations (three beds in the southern part of BHA; Fig. 1a) provide the only potential spawners since there are no known populations in the open sea. BHA is the major spat collection site for local mussel culture, but it is not used for mussel grow-out (Fig. 1a). Larval development and

trophic characteristics were monitored during the reproductive period, from May to August of 2007 and 2008. Whereas 2007 was an exploratory year in the project, we improved our effort in 2008 by integrating microscale spatial variability, by using higher resolution and by focusing on fine characterization of the trophic resources available for larvae only. Features of each sampling design were summarized in Table 1.

Larval production

The gonado-somatic index was calculated in adults (> 50 mm shell length) harvested from each mussels bed (Table 1; Fig. 1a). This index decreases rapidly during a major spawning event (Myrand et al. 2000). Larval concentration (individuals per liter) was assessed on seawater (1000 L) sampled from 0.5 to 2 m below the surface with a submersible pump and immediately sieved (80 µm square mesh; Table 1; Fig. 1a). The retained material was held in 80 µm filtered seawater on ice (within 6 hours) until veligers and pediveligers abundance was determined according to Aucoin et al. (2004) at a magnification of 100X (upright Olympus BX41, Olympus America Inc., Center Valley, PA, USA). Identified by their visible eyespot, pediveliger larvae are competent to settle and metamorphose into post-larvae (Fig. 1b). Larval shell length was evaluated by measuring randomly selected individuals (30 per sample) with an image analyzer system (CoolSnap Procolor and Image-Pro Plus v.5.0, Media Cybernetics Inc., Silver Spring, MD, USA) and size frequencies were calculated for four classes: i) < 130 µm represents new D-shape larvae, ii) 130–260 µm represents non-competent veligers, iii) 260–360 µm represents competent pediveligers (competency is acquired around 260 µm; McGrath et al. 1988) and iv) > 360 µm represents late pediveligers (Fig. 1b). In 2008, we estimated the proportion of byssal-drifting post-larvae that, characterized by the presence of a dissoconch shell and gills (Fig. 1b), are considered to be potential settlers (Lane et al. 1985).

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Settlement/recruitment

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We studied both settlement and recruitment on collector ropes (polypropylene; 1.27 cm in diameter and 1.33 m in length) suspended from a long-line typically used for commercial spat collection in order to prevent predation. Settlement rate (individuals per meter per day) was monitored by frequently sampling collector ropes that were immediately replaced by new ones (Table 1; Fig. 1a). In 2007 only, ropes were immersed for a period of two weeks and weekly sampled, thus inducing an overlap of one week for successive weekly deployments. Settlement rate was calculated by dividing the post-larval abundance on ropes by the immersion duration. Recruitment (individuals per meter) was studied by immersing a batch of 65 collector ropes at each station in mid-June and sampling them randomly on a weekly basis until the end of August (Table 1). These samples also integrate all post-settlement processes like hydrodynamics, physiological stress, predation, and competition (Hunt and Scheibling 1997).

Trophic resources

Seston quantity and lipid quality (fatty acids) were assessed on seawater (2 L per analysis) sampled at a depth of 0.5–1 m with a submersible pump and directly filtered through a square mesh sieve before storage in opaque bottles (Table 1; Fig. 1a). Mesh size was reduced to 20 µm in 2008 in order to match with maximal size of particles ingested by mussel larvae (< 25 µm; Raby et al. 1997), but also to provide quantitative and qualitative data consistent with results of flow cytometry (see below). Samples were then filtered on glass-fiber filters (GF/C, Whatman Ltd, Maidstone, UK) and total particulate and particulate organic matter (TPM and POM, milligram per liter) quantified according to Aminot and Chaussepied (1983). Fatty acids (FA) were extracted through direct transesterification (Lepage and Roy 1984) and analyzed on gas chromatograph (see Appendix A1 for technical details) to determine the mass of total fatty acids

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(MTFA; microgram per milligram of POM) and FA composition (%). In 2008, the planktonic composition of the seston was assessed by running samples (4.5 mL) on flow cytometer, which quantified six groups of < 20 µm planktonic cells (see Appendix A2 for technical details): heterotrophic bacteria were separated according to their nucleic acid content (LNA and HNA for low and high nucleic acid, respectively), and eukaryotes and cyanobacteria according to the pico (0.2–2 µm) and nano (2–20 µm) size classes (Belzile et al. 2008, Tremblay et al. 2009). Finally, temperature and salinity in 2007 and 2008 were recorded at a depth of 0.5–1 m at each sampling stations using a manual YSI 30/25 FT sensor (YSI Inc., Yellow Springs, Ohio, USA).

Data analysis

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We tested (i) the intra-annual variations of parameters by performing mixed-model repeated ANOVAs based on an autoregressive structure and subsequent pairwise comparisons, and (ii) the inter-annual variability through t-tests. The normality was verified by a Shapiro-Wilks test on residuals (i) or on raw data (ii), and homoscedasticity was tested by direct observations of residuals (i) or folded F tests (ii). When assumptions were not met, raw data were transformed, and if assumptions were still not met, conclusions were retained if they agreed with a rank test. Pearson's correlations were calculated between each pair of variables, particularly those including the settlement rate. A stepwise regression model was computed for the 2008 data to isolate parameter(s) with the highest explanatory power for the variation in settlement rate. SAS v.9.00 software (SAS Institute Inc., Cary, NC, USA) was used for analyses.

Results and discussion

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Data are expressed relative to T_x, where x is the number of days following the first occurrence of mussel larvae in the water column (T_0), which was 16 May in 2007 and 19 May in 2008. For each result, the average value plus or minus the standard error (Mean ± SE) is provided. In the

case of inter-annual comparisons, we present the results as 2007 vs 2008. As expected, every monitored parameter exhibited significant temporal variations ($p < 0.0001$; see Appendix B: Table B1 for detailed results). During both years, two major spawning events occurred at the beginning of the season (T6–11 and T18–25 vs T7–14 and T21–28; Fig.2 a).

Larval production and settlement competency

The mean veliger concentration throughout the season was statistically similar for both years (6 ± 2 vs 4 ± 2 ind/L; Table 2) and characterized by three peaks of abundance in 2007 (T28, T42, and T49) and two in 2008 (T24 and T38, Fig. 2b). The maximum veliger abundances were observed during the first peak, with no significant inter-annual difference (27 ± 15 vs 39 ± 18 ind/L; Table 2). The major veliger peak was related to the second spawning event in both years (Fig 2a,b), suggesting a low larval survival rate or flushing events of the larvae during previous spawning event. Several veliger peaks were not statistically significant due to large variations (Fig. 2b), reflecting the high spatial variability either at the basin (2007) or at the scale of the mussel farms (2008), and suggesting an aggregative horizontal distribution at a very small scale. Larval dispersal could be driven by hydrodynamic forces induced by both tides and winds in this type of lagoon (Koutitonsky et al. 2002). Globally water currents did not exceed 20 cm/s within the settlement area, whereas water input/output could reach 50 cm/s in the channel. In fact, import/export of larvae could occur as 90 % of the water could be renewed with 9 to 20 days according to observed tidal amplitudes (unpublished data). Nevertheless, larval behavior could also modify the passive patterns of dispersion since larvae cannot be considered as inert particles (Shanks 2009), not even in strong upwelling region (Morgan et al. 2009).

The first pediveligers (i.e., competent larvae) were observed at T28 in 2007 and T31 in 2008 (Fig. 2c), and their mean seasonal concentrations were similar both years (1.0 ± 0.3 vs

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1.0 ± 0.4 ind/L; Table 2). Three peaks of pediveliger abundance were observed in 2007 (T49, T63, and T74) and in 2008 (T35, T52, and T66); again, some peaks were not statistically significant due to high local variability. As we observed with veligers, maximal concentrations were observed at the first peak, with no significant inter-annual difference (4.8 ± 2.3 vs 5.4 ± 2.6 ind/L; Table 2, Fig. 2c), and they occurred within 21 vs 11 days after the major peak of veligers in 2007 and 2008, respectively. The time series of the two larval stage concentrations were moderately vs strongly correlated when pediveliger data were staggered according to the respective previous delays (Rho = 0.56 vs 0.84, $p = 0.01$ vs < 0.0001). In accordance with the expected duration of the planktotrophic phase (1–4 weeks; Bayne 1965), these delays would represent the time for larvae to acquire competency. Thus, the inter-annual difference in the delays (10 days longer in 2007) would reflect slower larval growth in 2007.

During both years, the mean larval length was similar (206 ± 15 vs 196 ± 12 µm; Table 2) and pulses of new larvae (< 130 µm) at T14 and T25 in 2007 and at T14 and T28 in 2008 coincided with major spawning events (Fig. 2a,d). Significant variations in shell lengths between sampling dates occurred only in 2008 and probably resulted from regular inputs of new larvae at T42, T59, T70, and T81 (Fig 2d). While the usual recognized size for competency is 260 µm (McGrath et al. 1988), mean larval shell length had maximum values of 321 ± 7 µm in 2007 and 299 ± 14 µm in 2008. The presence of byssal-drifting post-larvae (Lane et al. 1985) contributed to the increase in the observed mean larval size. However, their abundances, only estimated in 2008, were low (from 0.01 ± 0.01 to 0.21 ± 0.11 ind/L) and their proportions never exceeded 33 % of the potential settlers (except at T70 when they reached 75 %). In fact, most large individuals were late pediveligers, strongly suggesting a delay of metamorphosis (e.g., Bishop et al. 2006).

Settlement-recruitment

There was no significant inter-annual difference between the mean seasonal settlement (26 ± 9 vs 31 ± 9 ind.m^{-1}.d^{-1}) and the maximum peak settlement rate at T75–82 in 2007 and T52–56 in 2008 (92 ± 64 vs 163 ± 22 ind.m^{-1}.d^{-1}; Table 2, Fig. 2e). Settlement rates comparisons between the two years seemed valuable despite the difference of immersion period (2 weeks vs 3–4 days). Collector ropes are not expected to be a limiting factor for settlement because such structures could sustain very high abundances of spat, as denoted by values obtained during the recruitment monitoring (see below). The 2007 peak did not differ significantly from values obtained during the previous weeks due to high variances. Such variability could again be a consequence of high local spatial heterogeneity in settlement, but also of the one-week overlap between two successive periods of rope immersion. In this context, the fact that the settlement rate decreased significantly after T82 suggests that the peak occurred rather at T68–75 than at T75–82 (Fig. 2e). Settlers appeared as soon as competent larvae were available in the water column, but abundances of both stages were not correlated (Rho = 0.33, p = 0.36 vs Rho = -0.14, p = 0.38). The settlement peak occurred only 19 or 17 days after the period of maximum pediveliger abundance in 2007 and 2008, respectively. Some authors have emphasized that it is risky to relate larval supply to settlement intensity (Miron et al. 1995, Olivier et al. 2000) and *vice versa* (Shanks 2009) since behavior is a major source of asynchrony (Pineda et al. 2010). However, there was strong evidence of a settlement delay for both years until specific cue(s) would trigger the end of the pelagic phase. The high mean larval size during the settlement peak (315 ± 16 in 2007 and 285 ± 5 µm in 2008) supports this hypothesis, as Martel et al. (unpublished data) report a gradual increase in the shell length at metamorphosis (prodissoconch II) throughout the reproductive season in the Bassin du Havre-Aubert.

Even though recruitment correlated with settlement rate only in 2007 (Rho = 0.68, p = 0.02 vs Rho = 0.34, p = 0.05), it was considerably improved by the peak settlement rate for both years, as revealed by the highest recruitment increase during these periods: 5 556 ± 1 076 (T68–75) in 2007 and 5 758 ± 745 ind/m (T50–57) in 2008. On the whole, the mean seasonal recruitment was poorer in 2007 than in 2008 (6 171 ± 1648 vs 13 395 ± 2 426 ind/m; Table 2, Fig. 2f) despite the very similar year-to-year dynamics of veligers and pediveligers in the water column and the same kinetics of local settlement. While the maximum recruitment was statistically similar for both years (14 938 ± 3 374 ind/m at T82 vs 26 331 ± 2 123 ind/m at T71; Table 2), the final recruitment (observed at the last sampling date) was still lower in 2007 comparatively to 2008 (9 768 ± 1 664 at T103 vs 16 066 ± 683 ind/m at T99; Table 1, Fig. 2f). The recruitment decline at the end of the season (after T82 in 2007 and after T71 in 2008) would result mainly from self-thinning processes relating biomass to density (Frechette and Lefaivre 1995), and also predation by seastars and fall-offs of epibiotic macro-algae (Bourque and Myrand 2007a,b).

A match/mismatch with trophic quality

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Trophic conditions were very similar for the two years, with statistically comparable concentrations of total (TPM; 3.0 ± 0.3 vs 3.5 ± 0.3 mg/L) and organic particulate matter in the seston (POM; 1.0 ± 0.1 vs 0.9 ± 0.1 mg/L; Table 1, Fig. 3a). The higher POM/TPM ratio in 2007 indicates better POM availability than in 2008 (40 ± 4 vs 30 ± 2 %; Table 2). The masses of total fatty acids (MTFA) in the seston did not differ statistically between years (31.9 ± 6.5 vs 29.7 ± 1.5 µg/mg of POM; Table 2, Fig. 3b). These similar POM and MTFA contents in conjunction with a reduction of the sieve mesh to 20 µm in 2008 could suggest that organisms in the pico and nano size ranges dominated the planktonic communities in this lagoon.

Despite a remarkable year-to-year stability in seston quantity (POM and MTFA), its lipid quality differed drastically. Mean seston levels of saturated fatty acids (SFA) were higher in 2007 than in 2008 (71.3 ± 5.1 vs 39.6 ± 0.6 %), and consequently we found lower levels of monounsaturated (MUFA; 10.0 ± 1.8 vs 20.9 ± 0.7 %), polyunsaturated (PUFA; 18.7 ± 3.3 vs 39.6 ± 0.8 %) and essential fatty acids (EFA; 1.7 ± 1.7 vs 21.8 ± 0.6 %; Table 2, Fig. 3c). The planktonic food source seemed to be mostly heterotrophic in 2007, with low levels of PUFA and EFA, and autotrophic in 2008, with higher levels of PUFA and EFA (Sargent et al. 1999, Dalsgaard et al. 2003). The reduction of the sieve mesh size in 2008 could not explain such differences: phytoplankton is the main producer of EFA and if algal production had been consistent in 2007, EFA levels would have been higher through "trophic modification" by heterotrophic protists (Tang and Taal 2005, and references therein).

In 2008, the mean total abundance of bacteria was $3.7 \pm 0.1 \times 10^6$ cells/mL and the bacterial compartment was dominated by cells with high nucleic acid content (HNA; accounted for 61–75 %) exhibiting a mean abundance of $2.5 \pm 1.0 \times 10^6$ cells/mL (Fig. 4a). Abundances of HNA and total bacteria were strongly correlated (Rho = 0.98, $p < 0.0001$). Abundances of pico- and nanocyanobacteria exhibited seasonal means of 7 994 ± 1 353 and 38 ± 6 cells/mL, respectively (Fig. 4b), and were correlated (Rho = 0.54, $p < 0.0001$). The pico- and nanoplanktonic communities were dominated by eukaryotes (99.7 and 85 %, respectively). Abundances of pico- and nanoeukaryotes exhibited seasonal mean of 44.5 ± 4.0 and $14.5 \pm 1.2 \times 10^3$ cells/mL, respectively (Fig. 4c), but were not correlated (Rho = 0.07, $p = 0.4$). With its very low concentrations of inorganic nutrients (< 1.1 µM for nitrogen and phosphate and < 3.9 µM for silicate, unpublished data) and its high abundance of bacteria, the present lagoon

269 can be classified as oligotrophic with a bacterial-based food web. This classification was
270 suggested by Trottet et al. (2007) for another lagoon of the Îles de la Madeleine archipelago.
271 These authors also reported that heterotrophs dominated such lagoons and that autotroph blooms
272 were always short and very occasional, as we observed in 2007. However, the mostly autotrophic
273 production in 2008 tempers these finding and emphasizes the importance to using flow
274 cytometry to quantify picoplankton rather than relying on microscopic observations alone.
275 The low EFA content in the 2007 seston could explain the lower larval growth rate (Delaunay et
276 al. 1993), but the year-to-year similarity of the settlement intensity suggests that larvae still
277 accumulated sufficient lipid reserves to meet the high energetic demand of metamorphosis
278 (Videla et al. 1998, Pernet et al. 2004). These results suggest that even heterotrophic production
279 poor in EFA could sustain a standard larval cycle. Thus, this raises questions about previous
280 experimental results, which focus on the EFA requirement in larval development (Soudant et al.
281 1998, Glencross 2009). In fact, bivalves could overcome a dietary EFA deficiency by *de novo*
282 biosynthesis of non-methylene interrupted PUFA (Zhukova et al. 1992). However, the low lipid
283 values of the seston may have influenced the recruitment success observed in 2007, since
284 juvenile performance depends directly on the trophic conditions experienced by larvae (Phillips
285 2002, 2004). The higher recruits survival in 2008 compared to 2007 (Fig. 2f) may be related to
286 the higher seston EFA levels that year (Fig. 3c). In fact, the lower recruitment in 2007 could
287 result from a mismatch with a low EFA content in the trophic resource, suggesting that the
288 Cushing's match/mismatch theory (1990) is applicable with the food lipid quality. That
289 strengthens the importance of considering food quality in recruitment studies (Vargas et al. 2006)
290 in addition to food abundance (Durant et al. 2005) and composition (Beaugrand et al. 2003).
291 A trigger for settlement 2nd-order head

In both 2007 and 2008, the main mussel settlement peak coincided with specific conditions in the seston that could provide conditions enhancing settlement rate. In 2007, despite the absence of a correlation between seston components (TPM, POM, MTFA, SFA, MUFA, PUFA, and EFA) and settlement rate, the settlement peak (T68–75; Fig. 2e) followed a phytoplankton bloom (T67), which was drastically different from the overall heterotrophic conditions observed throughout the season. This pulse was reflected by the highest values of POM concentration (1.7 ± 0.2 mg/L; Fig. 3a), availability (POM/TPM > 50 %), and related MTFA (69.1 ± 8.4 µg/mg of POM; Fig. 3b). Seston fatty acids profiles revealed a mostly autotrophic plankton production at T67, with lower levels of SFA (41.3 ± 2.5 %) and greater proportions of MUFA (20.8 ± 0.8 %), PUFA (38.1 ± 2.7 %) and EFA (11.7 ± 2.8 %; Fig. 3c).

In contrast to 2007, there were no major changes in the seston quantity and lipid quality in 2008: the maximum POM value (1.5 ± 0.4 mg/L) was measured on the last sampling date; the POM/TPM ratio exceeded 0.5 only at T59, after the settlement peak, and MTFA ranged from 15.7 ± 3.0 (T42) to 43.0 ± 9.3 µg/mg of POM (T17; Fig. 3a–b). During all of the 2008 sampling season, FA profiles were similar to T67 in 2007, thus reflecting a continuous autotrophic production (Fig. 3c). Fatty acids groups exhibited slight but significant temporal variations, but only MUFA were correlated with the settlement rate ($Rho = 0.44$, $p = 0.02$, Fig. 3c). In contrast, the settlement peak in 2008 (T52–56) was synchronized with a pulse of picoeukaryotes reaching a maximum concentration of $133.4 \pm 5.4 \times 10^3$ cells/mL at T56 (Fig. 4c). In fact, the picoeukaryote abundance best correlated with settlement rate ($Rho = 0.57$, $p < 0.0001$), which was also significantly related to the nanocyanobacteria concentration ($Rho = -0.32$, $p = 0.02$) and the proportion of HNA bacteria ($Rho = 0.45$, $p = 0.0009$).

Among the 19 variables integrated in the regression model, three explained 83 % of the settlement rate variability: picoeukaryotes had the highest contribution ($R^2 = 0.59$) followed by nanoeukaryotes ($R^2 = 0.19$) and picocyanobacteria ($R^2 = 0.04$; see Appendix B: Table B2 for detailed results). We thus hypothesize that a change in seston characteristics triggered settlement and that this 'trophic settlement trigger' depended on both resource quantity and quality. For both years, the increase in resources rich in EFA would constitute high-value food, providing suitable conditions for larval metamorphosis and post-larval survival. Since Courties et al. (1994) demonstrated that picoeukaryotes could dominate the phytoplankton biomass in a coastal lagoon, it has been thought that they could play an important ecological role in such environments (Bec et al. 2005). In addition, the high content in dietary EFA biochemically benefits larvae during metamorphosis (Soudant et al. 1998, Pernet and Tremblay 2004) and post-larvae during benthic life (Phillips 2002, 2004).

The phytoplankton succession is controlled by hierarchical physical factors such as water motion, irradiance, and temperature (Levasseur et al. 1984), and picoplankton is also subjected to these controls (Winder 2009). In the present study, the trigger occurred during a short temporal window, when seawater was warmer than 19 °C and the salinity was 30–31 ppt (Fig. 3d); such conditions could indirectly influence settlement dynamics by conditioning plankton production. There was no significant inter-annual difference in the mean seawater temperature (15.0 ± 0.9 vs 16.3 ± 0.9; Table 2, Fig. 3d), which was only correlated to settlement rate in 2007 (Rho = 0.90, $p = 0.0003$). This parameter influences the metabolic activity of the plankton (Levasseur et al. 1984) and ultimately acts on biological processes, as observed for the recruitment success of the shrimp *Pandalus borealis* (Ouellet et al. 2007). In addition, warm temperatures could improve diet assimilation by enhancing cilia movement and the catalytic activity of digestive enzymes

(Rico-Villa et al. 2009). Water salinity, which was slightly higher in 2007 (31 ± 0.2 vs 30 ± 0 ppt; Table 2, Fig. 3d) was correlated to settlement rate only in 2008 (Rho = 0.52, p = 0.0001), but overall was not restrictive for larvae: the optimal salinity has been demonstrated to exceed 20 for *M. edulis* larvae (Qiu et al. 2002). Nevertheless, salinity variations could reflect freshwater inputs and could influence biological processes through plankton production, as demonstrated by Starr et al. (1993) on the spawning of the green sea urchin, *Strongylocentrotus droebachiensis.* In fact, the settlement peak occurred after a significant decrease in salinity in 2007 (Fig. 3d), as a consequence of heavy rainfall (70 mm in 3 days). Finally, the planktonic production could also depend on the water masses imported from the open sea. Further studies have to be conducted to understand the mechanisms involved in such synchronization since the trigger could also be related to the release of chemical cues associated with phytoplanktonic exoproducts (Starr et al. 1990) and/or to biofilm (Hadfield 2011).

Conclusions

In heterotrophic conditions (2007), we validated our hypothesis (H1) relating mussel settlement to levels of essential fatty acids (EFA) in the seston. However, in autotrophic conditions (2008), no such relationship was observed, probably because other food cues were involved in the settlement process. Nevertheless, our results imply that settlement peak would be triggered by a pulse of autotrophs irrespective of the trophic environment throughout the season. In addition, we validated our second hypothesis (H2) linking recruitment success to a match/mismatch with the seston EFA at the scale of the reproductive season: a mostly autotrophic production with abundant EFA results in higher mussel recruitment than a heterotrophic production with higher saturated fatty acids. For the first time, we suggested not only a bottom-up influence of the planktonic production on settlement, but also of the trophic lipids quality on recruitment in a

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marine bivalve. The two processes seem to act independently of each other, and the 'trophic settlement trigger' to be complementary to the match/mismatch by substantially improving recruitment success when present. While the potential advantage of trophic EFA reinforces laboratory findings, further experimental studies under controlled conditions are needed to demonstrate this 'settlement trophic trigger' effect and to understand underlying mechanisms.

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521 Ecological Archives material

522 Appendix A: Methodological technical details for 1) fatty acids, and 2) flow cytometry analysis.

523 Appendix B**:** Detailed statistical results of the temporal variations analysis (Table A1), and the

524 stepwise selection of the regression model (Table A2).

525

526 Table 1: Description of the sampling strategies conducted in the Bassin du Havre-Aubert in 2007

527 and 2008.

Sampled parameters	2007	2008
Adults		
station (replicate)	3 (10)	3 (10)
frequency	weekly	weekly
Larvae		
station (replicate)	8 (1)	3 (3)
frequency	semi-weekly	semi-weekly
Settlement		
station (replicate)	2 (5)	3 (3)
frequency	weekly	semi-weekly
immersion	14 days	3-4 days
Recruitment		
station (replicate)	2 (5)	3 (3)
frequency	weekly	weekly
Trophic resources		
station (replicate)	4 (1)	3 (3)
frequency	weekly	semi-weekly
mesh size	80 µm	20 µm
planktonic composition	no	yes
Temperature / salinity		
station (replicate)	8 (1)	3 (3)

frequency	semi-weekly	semi-weekly

528

529 Table 2: Results of t-tests comparing 2007 and 2008 data in the Bassin du Havre-Aubert.

Dependent variables	t	df	p
Veliger concentration			
sm	-0.38	46	0.7070
max	-1.28	9	0.2315
Pediveliger concentration			
sm	0.51	30	0.6105
max	-0.17	9	0.8675
Larval shell length			
sm	0.58	47	0.5674
Settlement			
sm	-1.1	13.1	0.2917
max	-1.28	3	0.2907
Recruitment			
sm	-2.42	21	**0.0248**
max	-3.06	3	0.0552
final	-4.14	3	**0.0256**
Seston			
TPM	-1.24	40	0.2225
POM	0.53	40	0.5998
POM/TPM	2.36	40	**0.0231**
MTFA	0.01	7.3	0.9891
SFA	10.55	26	**<0.0001**
MUFA	-7.48	7	**0.0011**
PUFA	-6.23	6.8	**0.0005**

EFA	-14.27	26	**<0.0001**
Environment			
Temperature	-0.99	51	0.3275
Salinity	4.27	37.3	**0.0001**

sm = seasonal mean; max = maximum; final = end of August

TPM and POM = total and organic particulate matter;

MTFA = mass of total fatty acids;

SFA, MUFA, PUFA, and EFA = saturated, monounsaturated, polyunsaturated, and essential fatty acids;

Bold p-value indicate a significant difference (α-level = 0.05).

530

Figure legends

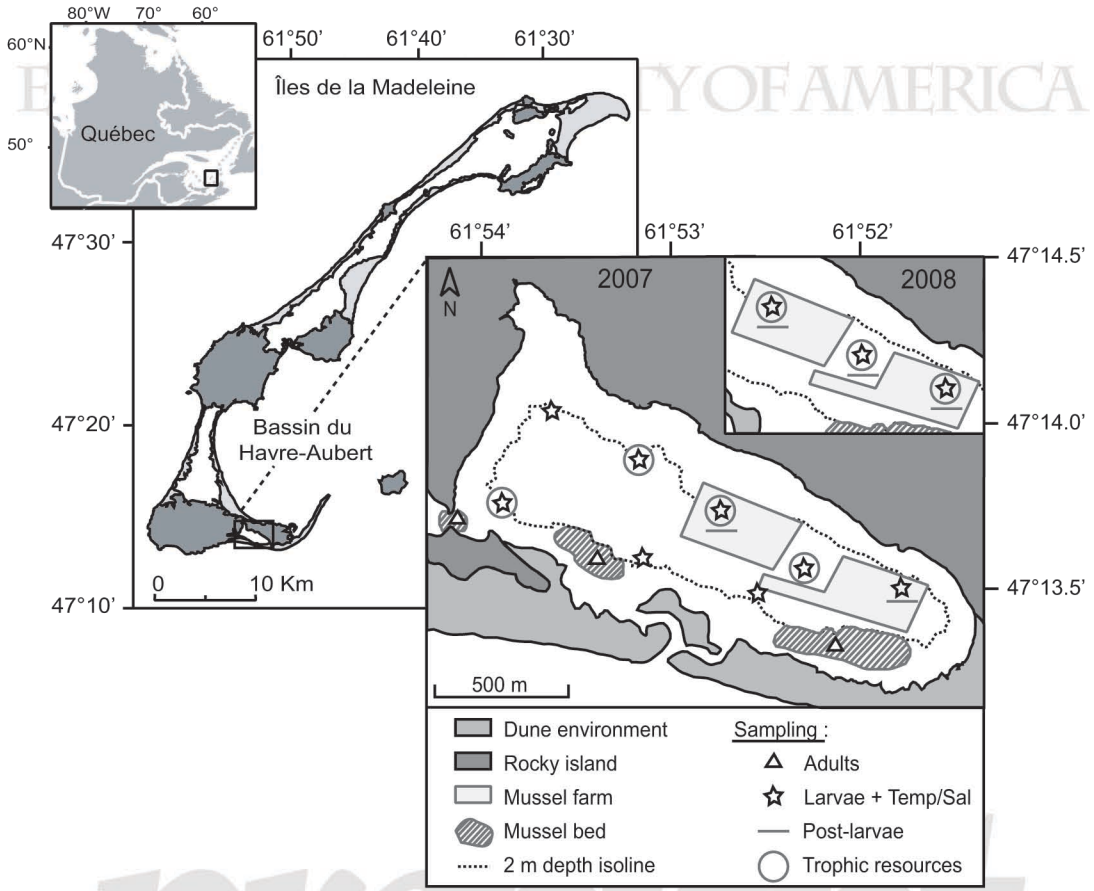
Figure 1: a) Location and map of 'Bassin du Havre-Aubert' (BHA) showing mussel farms, the natural mussel beds, and the 2007 and 2008 sampling stations. Temp = temperature, Sal = salinity. b) Developmental stages of young mussels with veligers and pediveligers larvae and post-larva.

Figure 2: Monitoring mussel larvae and post-larvae in 2007 and 2008: a) gonado-somatic index, b) and c) veliger and pediveliger concentrations, d) mean larval shell length (line) and related size frequencies (bars), e) settlement rate, and f) recruitment. Mean ± SE (n = number of samples, dotted line = seasonal mean). Arrow indicates a major spawning event and * a significant variation between two successive dates ($p < 0.05$).

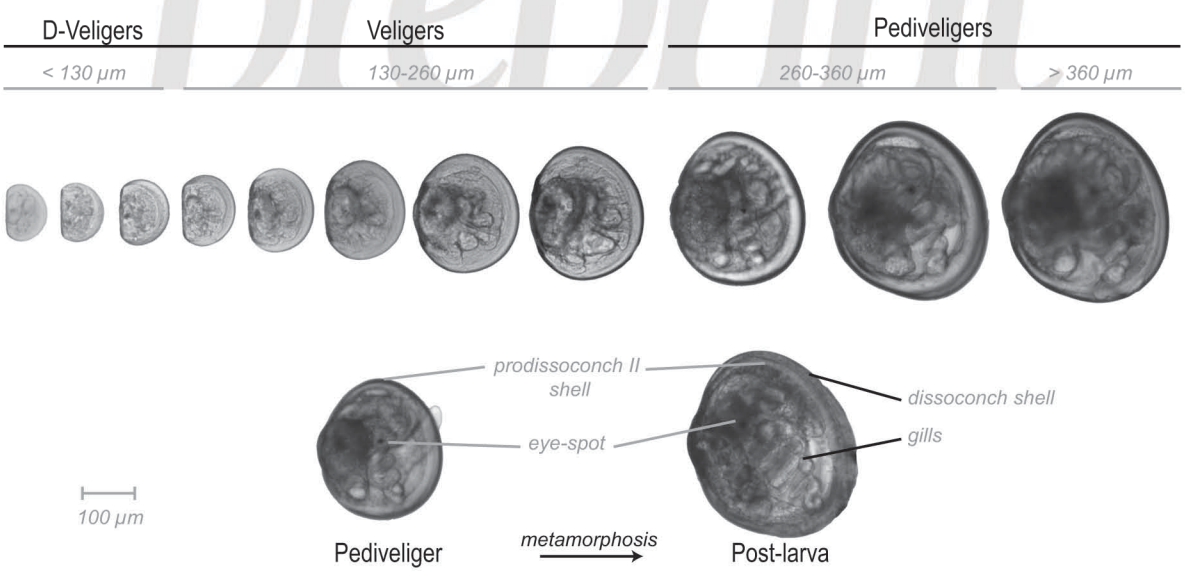
Figure 3: Monitoring seston and environmental conditions in 2007 and 2008: a) total particulate (TPM) and organic (POM) matter concentrations, b) and c) seston fatty acid content and composition, and d) water temperature (T) and salinity (S). SFA, MUFA, PUFA, and EFA = saturated, monounsaturated, polyunsaturated, and essential fatty acids. Mean ± SE (n = number of samples, dotted line = seasonal mean; stippled bar = mussel settlement peak). * indicates significant variations between successive dates ($p < 0.05$). For seston fatty acid composition (c), a, b, and c indicate significant differences for the associated fatty acids between successive dates.

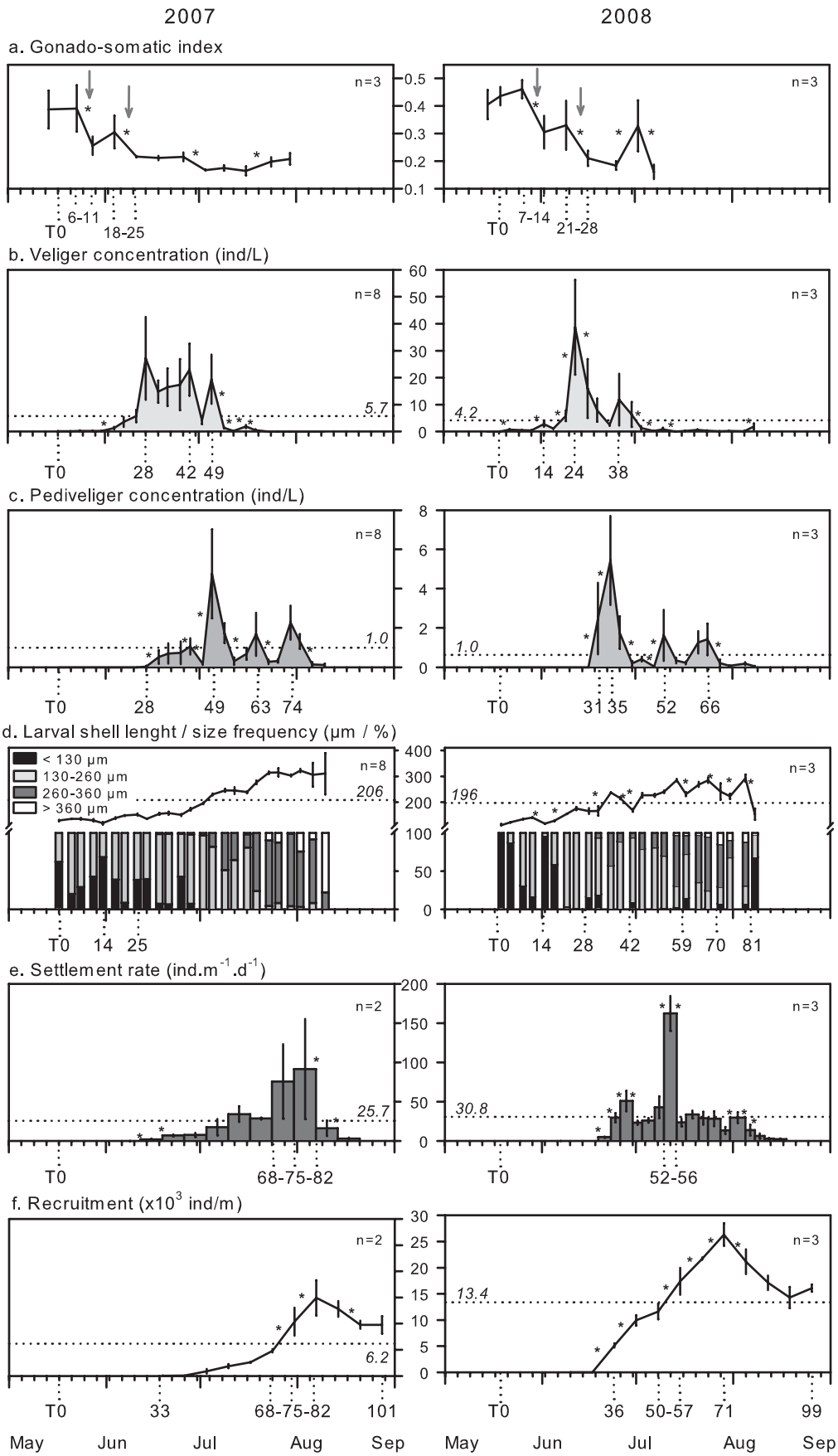
Figure 4: Monitoring concentrations of plankton components in 2008: a) total and high nucleic acid (HNA) bacteria, b) cyanobacteria, and c) eukaryotes. Pico- (0.2–2 µm) and nano- (2–20 µm) size classes are distinguished in b) and c). Mean ± SE (n = number of samples, dotted line = seasonal mean, stippled area = mussel settlement peak). * indicates a significant variation between two successive dates ($p < 0.05$).

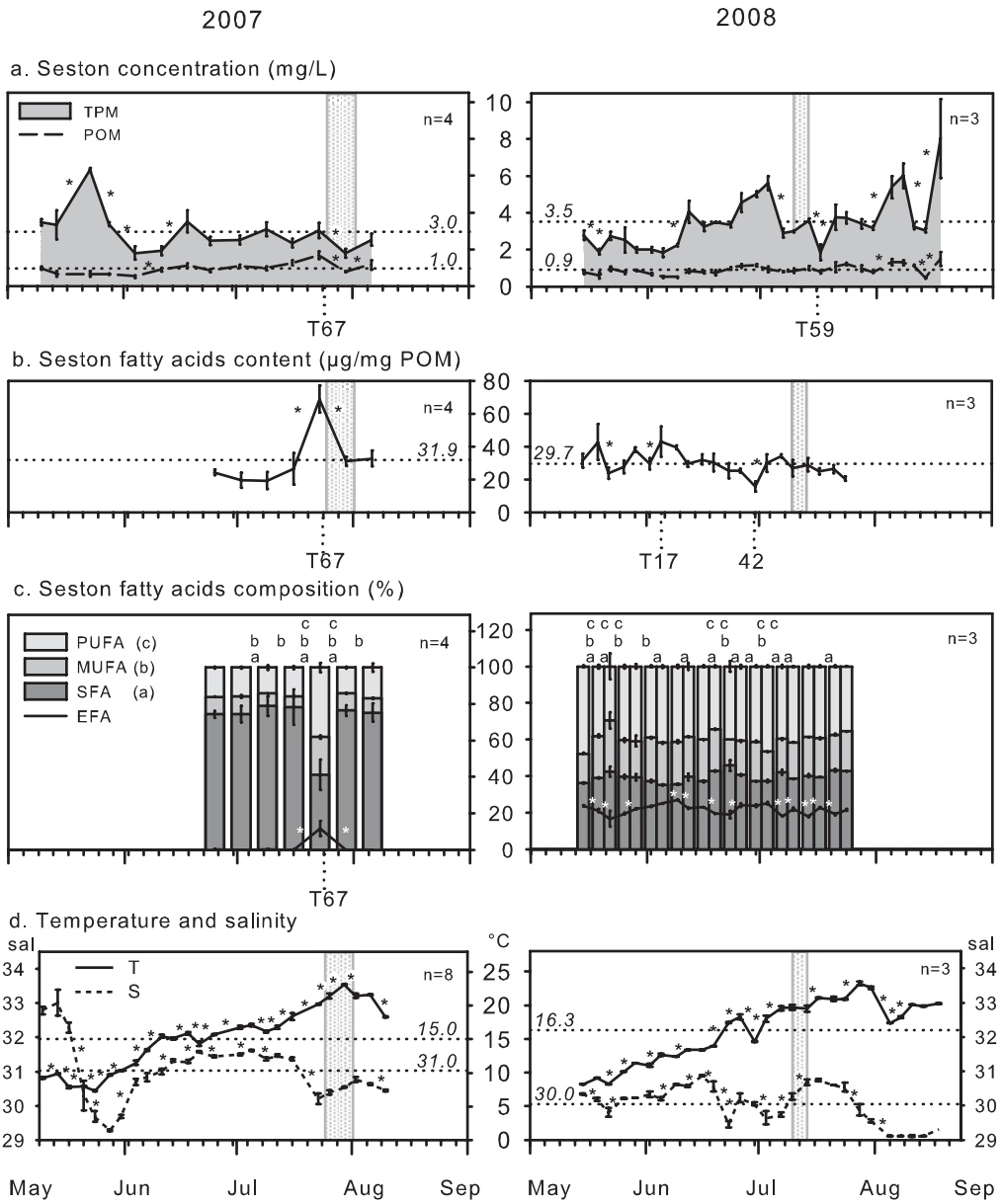
a. Study site



b. Developmental stages of mussel larvae







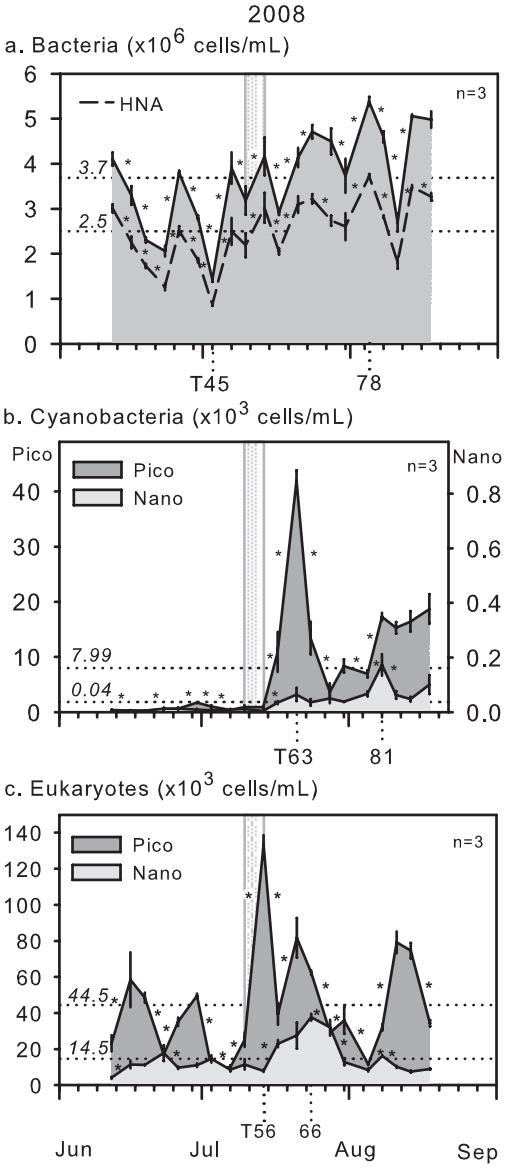


Plate 1. Large pelagic pediveliger larva of Mytilus edulis (380 lm) observed at a 1003 magnification. Photo credit: N. Toupoint.