CHAPTER 8

Summary and Discussion
IDENTIFICATION OF BIVALVE LARVAE

Adult bivalves are easily identifiable to species level by the characteristics of their shell. Despite the morphological and ecological differences of the adults, it has proved to be very difficult to devise an optical technique to identify larval species.

There are species-specific ranges for size of D-larvae, which make e.g. the youngest oyster larvae \textit{(Crassostrea gigas)} distinguishable from other species. No other obviously D shaped larvae are found between 70-75 µm length. D-larvae of \textit{Mytilus edulis} tend to be have a smaller width/length ratio than other larvae. Although the specific sizes of D-larvae and competent larvae differ between species and slopes are different, regression lines overlap and because we cannot say how old larvae are, it is impossible to make a distinction between species based on size alone.

Multidimensional scaling allowed us to see the larval shape develop from D-veliger into older, and subsequently larger, larvae. This transgression looks species specific.

Unfortunately it was not possible to separate all size classes of larvae according to species. Discriminant analysis on larger individuals (length > 150 µm) was relatively successful for those species for which sufficient individuals were available. Up to 74% of the larvae could be predicted correctly. Specialists can also make the distinction between species fairly accurately above this same size limit. Still there are advantages in further developing this shape parameterisation and computer analysis into a standard procedure. Once a proper procedure is developed, larger samples could be processed on a relatively short timescale without specialist knowledge.

Once again, an interesting question was raised after this study into larval shapes. Even with modern techniques, (young) larvae appear very similar in shape. It would be interesting to delve deeper into the question why the shape of young larvae is so conservative. Bivalve larvae are between 50 - 400 µm in length and swim/sink at Reynolds numbers in the creeping flow range. At this hydrodynamics regime shape does not influence the drag coefficient. Only a significant change in surface-to-volume ratio would affect total drag on the body and hence swimming / sinking velocities. So, large protruding structures, such as spines would have an effect, but subtle changes in shell shape not. Therefore we cannot suppose that hydrodynamic optimisation is the driving force behind the uniformity in shape of bivalve larvae. There must be other causes at work than hydrodynamic limitations to preserve the larval form in its present state, e.g. a strong phylogenetic load or other selective pressures.

Influence of food quality and quantity on larval development

Our experiments indicate that food quality of adult bivalves has a large influence on breeding success, while the effect of enrichment of the larval diet was minimal. Keeping adult \textit{Macoma balthica} in the laboratory at low temperatures to extend the spawning season caused lower fecundity (egg size and number), compared to directly spawned individuals irrespective of diet. However, deterioration of fecundity was less when females were fed an enrichment diet with added polyunsaturated fatty acids (PUFAs). For future (spawning) studies, we should always aim to feed an optimum diet to prevent gonad resorption in \textit{Macoma} adults. This study has implications for field studies as well. We showed that monitoring chlorophyll content is not sufficient to predict reproductive output of \textit{M. balthica} and development (and metamorphosis potential) of their larvae. Not only food quantity matters but also quality. Because algae species differ in PUFA content it is important which algae are available as food. Culturing or condition algae in the laboratory cause PUFA content of the same species of algae to vary. A study into optimum
culturing conditions to obtain an optimal PUFA content of algae used as food would be helpful. An inventory of PUFA content of naturally occurring algae would help to better predict good or bad years for shellfish recruitment and thus be a tool in developing a sustainable shellfish fishery.

We did not find a positive effect of PUFA supplementation on larval growth, development or mortality. *Macoma* larvae in cultures fed only fresh algae were largest, followed by larvae in the supplemented culture. *Macoma* larvae appeared very sensitive to added lipids in the culture medium. This lipid emulsion possibly may have been harmful for the larvae. Due to this by-effect we were not able to evaluate the effect of enriched diets on growth and development of *Macoma* larvae.

Oyster (*C. gigas*) did not exhibit the same sensitivity to the lipid emulsion. There was no difference in mortality or growth between the control group fed with the cultured algal diet and the groups with added ICES lipid emulsion. No positive effect for PUFAs was found either. This could be an artefact due to presumed unsuitability of the lipid emulsion. Or protein content of the diet could have been a limiting factor in structural growth and development.

However, food quantity for larvae may well be a determining factor for the survival of larvae in the field. Temporal differences in larval peaks between sampling sites in Northwest Europe are caused by temporal differences between spawning. Spawning of bivalves is probably co-ordinated by a trigger like food availability. Other factors are a reaction to a temperature shock or gradient and possibly coordination by tidal elevation.

The most important contributor to total phytoplankton energy content in the field was ‘nanoplankton’. Comparing the maximal assimilation rate of an individual larva with the available palatable energy in the water showed that a larva never achieves maximal energy intake and thus can never grow at maximum growth rate. At most stations, the energetic content of the phytoplankton was sufficient to meet maintenance costs. However, in the Sylt-Rømø bight, larval maintenance costs were not met for at least half of the year. Bacteria do not add significantly to available food sources.

**Settlement of bivalve larvae, the issue of scale**

After survival of the planktonic stage, larvae need to settle on the substrate before recruiting into the adult population. This settlement process is difficult to study in the field. Laboratory studies seem a good alternative since we have control over environmental parameters (temperature, controlled flow). We need to maintain similarity of scale if we ever want to compare our results in the flume to field situations. The most important scaling law, similarity of Reynolds numbers is comparable between our laboratory set-up and field sites, this makes a comparison between the flume and field sites possible. Another scaling parameter that is important for settlement studies is the ratio between advection and turbulent mixing in the water column (Péclet number). We manipulate this ratio by placing a grid perpendicular to the flow direction of the flume. This method is a valuable tool to increase the turbulence intensity throughout the water column in a controlled way. Introduced bottom roughness (oyster ridge) increases turbulence intensity in the lower part of the boundary layer but has little effect on turbulence intensity in the water column. This means that it is possible to adjust both turbulence intensities in the water column as well as in the boundary layer in a flume. Shear velocity generally changes when turbulence is artificially increased. The estimate of $z_0$ was related to the distance at which the grid was placed from the test section.

The introduction of a grid or oyster ridge, generating turbulence with different origin and magnitude, results in similar Péclet numbers. Turbulence affects (lowers) the Péclet number. The Péclet number in the flume under normal circumstances is relatively high compared to similar flow velocities in the field despite the fact that spectral analysis indicates that even at the lower flow velocity the flow in the flume
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is fully turbulent. Placing a grid at 3 meters in front of the test-section lowers the Péclet number to field values. The presence of the oyster ridge on the bottom lowers the Péclet number in the boundary layer. Higher values found for the Péclet number under normal flume conditions point to a bias towards sinking under laboratory conditions in the flume. The results of this study present evidence for the hypothesis that the settlement phase for weakly swimming bivalve larvae as well could be more dependent on active behaviour in the field than previously presumed according to flume studies. We hypothesise that, unlike in previous flume studies, in field circumstances turbulence and advection are roughly balanced and (vertical) swimming or deliberate sinking could make a difference in settlement probability for small bivalve larvae.

Processes that influence larval settlement take place on different temporal and spatial scales. The size of a channel used to investigate flow processes influences the properties of the flow (the channel Reynolds number). This is one of the foremost and most obvious scaling parameter that one takes into account for environmental fluid dynamics research. Turbulence influences the mean time it takes for a particle to settle on the bottom. This is another factor that should be taken into account for equipment used in this research. We have been able to manipulate these parameters successfully in a flume tank. However, if it takes a larva (or a passive particle) on average several minutes to travel through the water column, while the horizontal flow velocity is around 0.1 ms\(^{-1}\), then the horizontal scale of patch choice should be in the order of magnitude of tens of meters. This means that larvae will not be able to make a choice for primary settlement on patches that are only 0.3 m long. Virtually any flume tank would be too small to investigate this. Small-scale patterns in larval settlement must be the result of a smaller-scale process, such as:

- Processes in the viscous sublayer
- Secondary settlement

Near-bed flow

To study if there is a possibility to influence settlement in the viscous sublayer for bivalve larvae, we study near-bottom flow. A new method for near-bottom measurements (Particle Image Velocimetry; PIV) is evaluated against Acoustic Doppler Velocimeter (ADV) measurements. The ADV is a useful tool to measure flow velocities, but cannot measure close to boundaries rendering the technique unsuitable for near-bottom measurements. Close to the boundary, where the ADV is unable to measure, PIV measurements show lower flow velocities than expected from ADV profile extrapolations. The velocity gradient near the surface is very steep. Even under reasonably high flow velocities (0.15 ms\(^{-1}\)) and increased turbulence levels, we find an obvious viscous sublayer. Estimations of the thickness of this layer range between 1.5 and 10.8 mm depending on calculation method and flow velocity. The linear velocity profile of the viscous sublayer is clearly visible in a PIV velocity profile. A smooth two-parameter curve was fitted (\(r^2=0.90\)) describing a combined linear-logarithmic profile. We estimated at what length scale viscous forces become dominant (Kolmogorov length scale) using autocorrelation spectra at 10 mm height. The Kolmogorov length scale is estimated between 0.02 - 0.05 m. This is an order of magnitude higher than estimated \(\delta\), showing that these values are not comparable.

The results described in this chapter show that the PIV method is in principle suitable for near-bottom flow measurements and visualisation of the viscous sublayer. Observed differences in turbulence intensity in the logarithmic part of the boundary layer measured with an ADV seem to extinguish close to the substrate resulting in similar conditions with dominant viscous forces in near-bottom layers under different turbulent conditions in the water column.
Settlement of larvae in flow

PIV measurements indicate reasonably high shear stresses present close to the bottom. This could be an argument for the entrapment of larvae in the viscous sublayer. More PIV measurements closer to the boundary are necessary. Our PIV set-up could be much improved by a more careful positioning of the camera resulting in measurements closer to the sediment. In addition to PIV measurements direct observations and tracking of individual larvae under varying turbulent conditions would provide us with the best evidence. However, the high magnification required, combined with the large field of view that is desirable for tracking may prove to be a technical challenge.

Settlement of larvae and mimics is influenced by flow velocity, with increased settlement at higher velocities. Plotting settlement data against $K_z$ shows an optimum effect of turbulent mixing on settlement. At low mixing constants, there is a rapid increase in settlement up till the critical resuspension point, after which erosion causes loss of settled particles. Similar profiles were found for larvae and mimics. This indicates that probably active behaviour cannot influence primary settlement on the scales of our experiment.

We hypothesise in a conceptual model that the mechanism causing increased settlement at higher flow velocities is increased turbulence. This model can be used to investigate to what degree larval behaviour may influence settlement patterns, and to interpret the difference between observations in still water (selectivity for sediment types) and in flumes (passive settlement). Two model interpretations are worth emphasising. Firstly, it seems likely that different behavioural adaptations are each only effective in certain sub-ranges of turbulence intensity. Adjusting sinking speed to increase settlement rate becomes very ineffective at high turbulence intensity, since under these conditions random movement overtakes advective sinking. Adjusting resuspension probability requires relatively high turbulence levels, since in very calm water resuspension is unlikely, no matter what a larva does to increase its probability of being resuspended. Also when excreting a byssus thread, a minimum drag on the thread is required to result in effective resuspension. However, combining both methods may give larvae an extensive range of hydrodynamic conditions where they may effectively influence their settlement probability. Secondly, the model permits to estimate orders of magnitude of the spatial scale over which larvae may react to cues in the sediment. Larvae reacting to sediment cues by stopping their swimming and thereby increasing their sinking velocity from approximately zero to somewhere in the order of 1 mm s$^{-1}$, would require a time in the order of $H/s$ (water depth divided by sinking velocity) to reach the sediment. Multiplied by the advective horizontal velocity of the water, typically $0.1$ m s$^{-1}$ in low-turbulence conditions suggests a spatial scale in the order of tens to hundreds of meters for an effective response. This spatial scale may be small enough to react to patterns in typical estuarine sediments; however it is much larger than the spatial scales that can be used in flume experiments. In our experiments both mimics (spheres and eggs) and larvae show a similar pattern in settlement. We suggest that laboratory flumes are inappropriately scaled to resolve phenomena of active selection of habitat, and may therefore falsely suggest that settlement of bivalve larvae is primarily a passive, hydrodynamic, process.

Upward swimming through the water column is an often-observed behaviour by which larvae become near neutrally buoyant particles. However, if turbulent mixing makes the larvae tumble and overturn, it is questionable whether they can keep their swimming speed directed upwards. Such shear-induced torque may spin the animals, causing them to swim haphazardly rather than towards the bed or upwards. Our model analysis suggests that this loss of behavioural influence on sinking speed at high levels of turbulence may not be crucial for the animals, since sinking speed loses its influence on settlement rate at these high turbulence levels anyway. Appropriate swimming behaviour is only important for larvae in relatively still water. At higher levels of turbulence, behavioural influence on resuspension (by secreting byssus threads) and thus secondary settlement seem to be the only effective mechanisms. Byssus drifting is a well-
described mechanism by which several bivalve species are able to influence migration. There appear to be species-specific differences in secondary settlement patterns, which would imply that different species would have different ranges of turbulence within which they are capable of influencing resuspension.

Further research would critically depend on the combination of large-scale field experiments and small-scale flume experiments (viscous sublayer). In order to test the influence of behaviour on settlement probability in the field, large patches of sediment should be experimentally manipulated. We suggest that scales of tens to hundreds of meters would be needed, corresponding to the scales over which natural settlement may vary.