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The implementation of laboratory studies to shrimp recruitment modelling - a brief review of experimental procedures

By

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Abstract

Shrimp has pelagic larvae and the time and location of larval settling are critical components for a successful recruitment to the population. Shrimp larvae occur in the surface layers after hatching and are then found in deeper waters until they finally settle. It is, therefore, essential for recruitment modelling to include robust data concerning the duration of the pelagic stages and the environmental factors that govern the vertical distribution of larvae. Laboratory experiments have given in-sights to the growth performance and duration of the various pelagic stages of shrimp larvae. The preference of a specific larval stage to given temperature, salinity and light regime has been successfully studied, in addition to feeding behaviour and responses to predators. Here we present some designs and techniques that have been employed for the study of shrimp larvae in the laboratory.

Key words: Shrimp, larvae, behaviour, experiments

Introduction

The shrimp (*Pandalus borealis*) is a protandric hermaphrodite that changes sex at an age of 4 to 7 years in the Northeast Atlantic (Nilssen and Hopkins, 1991). The shrimp spawns in autumn and ovigerous females carries out-roe until spring when the larvae hatch. Within a period of 2-3 months the shrimp larvae pass through seven developmental stages after which they settle (Shumway *et al.*, 1985).

It is of major importance for stock assessment of shrimp to gain information on the strength of the recruiting year classes as early as possible. Today's assessment forecast of the shrimp stock productivity and potential fishing yields is weak partly due to lack of knowledge on the population dynamics from hatching and until the shrimp enter the fishery. According to Shumway *et al.* (1985) the year class strength of shrimp is largely established during the pelagic larval stage. It is believed that transport processes influence recruitment both directly as advectional losses of larvae and indirectly through temperature, food availability and predator-prey interactions (Apollonio *et al.*, 1986; Clarke *et al.*, 1991; Lysy and Dvinia, 1991; Ouellet *et al.*, 1995; Anderson, 1999).

Current fields from a hydrodynamic model for the Barents Sea have been fed into a Lagrangian particle-tracking model to simulate the transport of shrimp larvae. The advection of the shrimp larvae is driven by the physical component, while a biological model will describe the vertical position of the shrimp larvae. However, the lack of accurate biological data on shrimp larvae limits the modelling work. Robust data on larval hatching, development and behaviour obtained from laboratory experiments will be used as input data in models (Ådlandsvik and Sundby, 1994; Hansen and Ådlandsvik, 1996; Pedersen *et al.*, 2000). This paper identifies the knowledge gaps in shrimp recruitment modelling, presents some experimental designs for studying ecological preferences and behaviour of shrimp larvae, and review some earlier methods for rearing shrimp larvae in the laboratory.

Knowledge gaps in shrimp recruitment modelling

The input to the particle-tracking model is the regional distribution of ovigerous female shrimp, the date when 50% of the eggs have hatched in each area, the duration of the pelagic larval period and the vertical distribution of larvae until settling. Information on

the distribution and abundance of ovigerous females, the number of eggs and the time of hatching is obtained from field sampling. Nevertheless, information on the growth performance of the larvae throughout six zoea stages and the duration of the pelagic larval period is achieved from laboratory experiments (supported by plankton sampling in the field). As the tracking model includes information on temperature and salinity, knowledge about the stage specific larval responses, preference as well as mobility along gradients is needed. For example we need to know if newly hatched shrimp larvae zoea I (ZI) escapes the cold and fresh waters in the surface layers beneath sea-ice or die. Diurnal migration of shrimp larvae has been observed in the field and this may be included if information on responses to light enables us to create a pattern for diurnal migration.

In addition to temperature, salinity and light regimes a biological model will include aspects of feeding behaviour such as prey preference and feeding frequency and amount of food needed. Information concerning escape responses as well as the predation by fish larvae is also essential and cannibalism may occur when the larval density and size variability is high. We have not observed cannibalism in our experiments in lab but density related intraspecific interactions may be present. Information about environmental preference of shrimp larvae has been achieved through several laboratory experiments described below. The laboratory experiments conducted on shrimp larvae in this study are listed in Table 1.

How to obtain shrimp larvae

The technical application of laboratory experiments for providing in-put data for modelling includes several aspects of how to handle live shrimp and larvae. Sampling, transport, storage and feeding of ovigerous shrimp females will not be described in this paper. The holding tanks (100 l) for ovigerous females were kept clear of visible particles, oxygen levels above 8 mg/l, low temperature (2-8°C) and low water exchange rates (8 l/min). The shrimps were subjected to darkness or red light when handled.

Larvae were collected every 24 hours to avoid starvation and problems in identifying the time of hatching (Fig. 1). After collection of larvae in the female holding tanks, the tanks were exposed to overflow for at least 15 min to remove excess larvae.

Newly hatched larvae have positive photo taxis and a flashlight was used to gather the larvae. A wide glass jar (150-250 ml) was gently put into the water surface and larvae were captured within the current filling the jar. After sampling the larvae were counted, kept in darkness and at constant temperature until transfer to rearing tanks. For temperature experiments it is preferable to acclimatise in the brood-stock females before hatching takes place. Low temperatures may prolong the hatching period and reduce the number of larvae hatched per day.

Rearing of larvae

Presentation of approaches

We have conducted rearing experiments using various size and shapes of tanks. The number of shrimp larvae introduced to each tank also varied (Table 2).
It was important to offer the larvae good water quality, e.g. high salinity (33 ppt.), filtered and oxygenated water (no air bubbles). Water inflow was submerged to avoid air-bubbles. Outlets were constructed so that the tanks could be over flown and flushed during cleaning without affecting the other rearing tanks. All temperature changes affect the larvae so any temperature change was made gradually (max. 0.5°C when operating within the range 2-8 °C).

Growth and developmental experiments

In developmental experiments we registered larval stages and time to settling. For the growth performance measurements carapace- and total length in addition to dry weight were registered. In 1999, experiments were performed in 90 l incubators (Fig. 2a). These experiments did not turn out successfully as the mortality of ZIV and ZV was very high. The mortality was probably caused by lack of substrate/bottom to settle, or by unattended microflora as e.g. flexibacta blooms. Therefore 2,5 l compartments with flat bottom were introduced for growth studies in 2000 (Fig. 3). For general observations and holding of larvae we used 250 l rearing tanks developed for marine fish larvae with an automatic self cleansing system (rotating arm) (Fig. 2b).

Larvae obtained from ovigerous females sampled in Balsfjord, a fjord in the Northern Norway, and from Hopen, in the Barents Sea were exposed to two temperature treatments; *in situ* simulation of Balsfjord (4°C) and Hopen (2°C) waters. There were 20

compartments with 50 larvae in each, in both treatments (density: 1 larvae 50 ml^{-1}). All compartments had independent water inlet and outlet. Every 14 days 10 larvae were removed from each of the four set-ups. The light was dimmed constant red in order to reduce potential light- induced stress.

Food preference experiments

The larvae were reared in the large holding tanks (250 l), prior to the feeding experiments. All larvae, except ZI, were fed a mix of algae, newly hatched and enriched *Artemia sp.* naupli and natural plankton (<250 μm diam). Gut filling was easy to observe (Fig. 4).

Glass beakers (2 l) were filled until 1,8 l with oxygenated, 4 °C filtered (10 μm Millipore filtered) natural seawater. Ten starved larvae were added one at the time by a wide mouthed plastic pipette to each jar. The jars were then left in a cooled and dark room for 30 min for acclimatization. The different food items were pre-washed and diluted in micro-filtered seawater and added to the glass jars containing the larvae. Finally, the beakers were filled up to a total volume of 2 l. The larvae were then observed for gut filling every 90 minutes, up to 12 hours.

Overview of the different food items tested and the results is listed in Table 3. The temperature of the algae and the warm water *Artemia sp*.-naupli was adjusted prior to the experiment. If the food experienced a temperature shock, it would sink out and become unavailable to the shrimp larvae. The food preferences experiments indicate that shrimp larvae fed on all food items, but the food selection seem to be size dependent. The larvae in stage ZI and ZII have a higher clearance rate for algae than for other food items offered, while ZIII looses interest in planktonic algae. The shrimp larvae seem to need time to adjust to new food since they reacted by reduced consumption when new food was added. When the larvae had adjusted to new food the gut filling remained high. Shrimp larvae (stage ZIII-ZVI) actively chase and ate smaller capelin and cod larvae when they were introduced.

Shrimp larvae feeding on artificial semi-floating food did not survive to ZIII. The high mortality may be caused by low nutritional value of the food, clogging of extremities. The shrimp larvae in the rearing experiments were fed *ad libitum* with a mix of enriched

Artemia sp. naupli, *Chaetoceros* sp. and natural zooplankton (≤ 250 μm) in order to ensure an optimal diet. The growth and survival with such a mixed diet have proven good in our experiments. In order to obtain *ad libitum* food conditions and avoid excess food in the tanks, we adjusted the amount and type of food given to the shrimp larvae throughout the rearing period, by calculating the clearance rate of the different foods.

Behavioural studies; the Plexiglas Cylinder Observation Method (PCOM)

The responses of shrimp larvae to light, salinity, temperature, food and predators were studied using plexiglas cylinders. The larvae do not see the observer due to reflection in the walls of the cylinder and observation is easy due to the magnifying effect of the bended glass. Systematic positioning of the larvae is obtained by scaling the cylinder in stratified layers. The PCOM used were 1,20 m tall with a diameter of 20 cm (Fig. 5). Only ten larvae were introduced in each observation-series since the ability to register mobile larvae simultaneously is limited. Perhaps introduction of video recording would make observation of larger no. of larvae possible.

Response to light

The cylinder was filled with filtered seawater with a salinity of 33 ppt. and temperature of 4^{o}C. The water column was stirred before the larvae were added to ensure a homogenous water body. The larvae were acclimatised for 4 hours in darkness in the rearing tanks prior to the experiment. The movable light sources were filtered with black plastic film and the water column was divided into four sections of illumination (Table 4).

The ZI, ZII and ZIII larvae showed positive photo taxis. The ZIV larvae swam towards the brightest illuminated section, stay for some minutes and then swam towards less illuminated sections.

Response to salinity

A salinity gradient from 0 to 35 was established by using mixtures of filtered seawater and distilled freshwater (all 4 °C). The ZI and ZII larvae tended to stay in the section with a salinity of 28 ppt. (Table 5), where as ZIII and ZIV stayed in the sections with higher salinity.

Response to temperature

A temperature gradient from 5 to 0°C and with stable salinity (33 ppt.) was made filling up the PCOM with pre-cooled water and maintained by cooling the bottom section with ice. The larvae of all stages congregated in the layers of highest available temperature 4.6 °C. The activity of the larvae was reduced when exposed to low temperatures (0°C), but no mortality due to temperature shock was observed (Table 6).

Response to food

Homogenous temperature (approximately 4°C) and salinity (33 ppt.) throughout the column was established. *Artemia sp.* and algae were introduced at different sections at different light conditions. In all trials the shrimp larvae gathered in the layers with the highest food concentration available. Manipulation with salinity and light did not affect the feeding behaviour. However, the shrimp did not enter layers with low salinity (0-20 ppt.).

Response to predators

In order to study the response of the shrimp larvae to predators, we introduced ten larvae of the spotted catfish (*Anarhichas minor*) that had been fed shrimp larvae earlier. The cylinder was fully illuminated and the water temperature was approximately 4 °C and the salinity 33 ppt. The shrimp larvae ZI and ZII showed no obvious reaction when predators were introduced. The larvae kept their position near the light source, but when light was turned off the larvae spread out in the column. The ZIII and ZIV larvae did not show any reaction to the introduction of predators. However, they were more successful in escaping the catfish larvae when attacked. The catfish larvae sank out and stayed at the bottom during the dark period. This indicates that the catfish larvae are dependent on visual contact with their prey organisms. The larvae of spotted catfish is possibly not a natural predator of shrimp larvae in the field, but the only one available for studies in the lab at the time. The natural predator of pelagic shrimp larvae in field is probable krill and plankton- eating fish like juvenile cod and saithe, herring and capelin. The experiment should be preformed with other predators more frequent in the natural environment of shrimp larvae.

Discussion

Rearing of larvae

Several rearing experiments with shrimp larvae in laboratory have been performed and described (Table 7). Many studies have proven to be difficult to evaluate because the tanks and water quality have been only briefly described.

Tank size

The size of the tanks should be chosen according to the aim of the study. If the aim is to study larval development and moulting frequency, the size of tanks and number of larvae in each tank should be low (<20) (e.g.(Bøhle, 1977; Stickney and Perkins, 1981; Wienberg, 1982b; Nunes, 1984; Rasmussen, 1993), this to ensure the possibility to observe excuviae in the tanks. If studying survival, density and growth the tanks should be larger. The lager size gives room for larger no. of larvae and thereby the supply of sufficient sample sizes for statistical analysis. It is quite easy to maintain a constant environment in large tanks with a lower water flow than in the smaller compartments. The stable environment reduces stress and the larvae seem to thrive in larger water volume. Studies utilising the advantages of large tanks have been conducted previously (Saotome and Ikeda, 1990; Ouellet *et al.*, 1992).

Tank shape

Rearing tanks may vary in shape and color but they must have smooth surfaces that are easy to clean. Tanks for rearing shrimp larvae require a flat bottom after stage ZIII. The lack of a bottom in stage ZIV and older may cause mortality. The swimming behavior of the larvae seems to be reduced in stage ZV and the larvae were observed sitting on the bottom ”resting”, when not feeding off the bottom. This may explain the high mortality observed in the 90 l incubators that we used in 1999. Problems with tank shape are not discussed in the literature probably because experiments that failed are rarely published.

Feeding

Several food intake observations were made to ensure *ad libitum* feeding conditions for the larvae. Starvation of brachyuran crabs showed a particular sensitive period in the early zoea stages, and the occurrence of a P_{50} point-of-no-return for starvation (Anger *et al.*, 1981). Nunes (1984) found the same sensitivity to starvation in *P.borealis* larvae, but could not find that the point-of-no-return mechanism applied for them.

Almost all experiments with *Pandalus borealis* larvae have included *Artemia sp.* naupli as main source (Wienberg, 1975; Haynes, 1976; Bøhle, 1977; Haynes, 1979; Stickney and Perkins, 1981; Wienberg, 1982b; Nunes, 1984; Rasmussen, 1992). Stickney and Perkins (1981) listed diatom fragments as the most abundant food item in ZI and ZII

larvae from the field. In ZIII and ZIV crustaceans, polychaets, and unidentified invertebrate fragments were observed in the natural diet. Shumway *et al.* (1985) stated that both the adult and larvae of *Pandalus borealis* were omnivorous. Malnutrition may therefore be one of the most serious problems in rearing experiments performed and may have resulted in both unnatural development and survival strategies. By introducing algae and filtered zooplankton in addition to *Artemia sp.* naupli we seem to have obtained better nutritional conditions for the larvae. The use of artificial food was not successful in our experiments.

The best indicator of malnutrition in our experiments has been the lack of pigmentation of shrimp larvae fed artificial food, and of those fed solely on non-enriched *Artemia* naupli. The pigmentation in shrimp larvae food natural plankton and algae in addition to enriched *Artemia* naupli has been very close to the pigmentation pattern found in live shrimp larvae from plankton samples. All the conservation methods used for shrimp larvae remove pigmentation within hours, which makes the trial to crosscheck information about malnutrition in our experiments impossible. To provide natural food is important for the right nutrient composition of the food (e.g. lipids, vitamins and minerals).

Behavioural studies

The plexiglas cylinder observation method (PCOM) offer the shrimp larvae a more ”natural” environment for observation than traditional preference chambers. The PCOM makes use of natural mechanisms in the water column in order to provide different environment. The main problem is to divide the different environments, offered to the animals, as natural as possible, i.e. no physical barriers that the animals have to conquer. The effect of pressure was not tested, but for shrimp larvae, which have no swim bladder, this has been assumed to be negligible. Turbulence is another physical factor that may be relevant for the success of the shrimp larvae. Turbulence studies have been successfully conducted on herring larvae in a plexiglas cylinder were different levels of turbulence was introduced (Utne-Palm and Stiansen, 2000).

A horizontal version of the PCOM, which excludes the pressure depth (pressure) effect of the vertical PCOM chamber, is to be preferred in the study of marine fish larvae. The construction is the same as for the vertical PCOM but filling is then performed through

the inlets-vents. There is a problem with studying illumination preferences in the existing PCOM. Light is spread through the cylinder itself due to the fibre optical effect of glass cylinder. To avoid this the PCOM should be sectioned, and every section separated with a black glass or plastic ring. This will stop the light from spreading in the glass and the only spreading will be in the water column. We also tried a larger size PCOM that was 2,50 m tall and had a diameter of 50 cm. We introduced 100 larvae that proved to be too high since the observation registration took more than an hour to perform. The recognition of the accurate number of larvae in each section became difficult due to the movement of the larvae and the wide diameter of the PCOM. The advantage of the large PCOM is the possibility to prolong the observation series due to water volume with almost constant oxygen and temperature levels throughout the observation period.

Quality control of data

Experimentally obtained data always run a risk of presenting abnormal development due to laboratory artefacts (Krebs, 1985). The laboratory artefacts in the case of rearing shrimp larvae in the laboratory ranges from water quality (agents, salinity, amount of dissolved oxygen, pollutants), light, stress due to disturbance and handling, food composition and feeding procedures etc. The comparison of reared larvae and larvae from natural plankton is therefore the best quality check available on the methods used.

The developmental time and number of stages of the pelagic shrimp larvae of *Pandalus borealis* is in literature reported to vary between 45- 120 days and from 6 to 11 stages (Shumway *et al.*, 1985). Data presented by Stickney and Perkins in (Shumway *et al.*, 1985) gives a developmental time for larvae in the Barents Sea may last 90 to 120 days or more (<4°C). This is one month of difference in time of settling. In addition low temperature environments like the Barents Sea have been expected to be too cold to sustain the growth and development of shrimp larvae, which have an optimal temperature of 3 and 9°C (Lysy, 1982; Shumway *et al.*, 1985; Lysy and Dvinia, 1991). The Thermal Increase Enhancement (TIE) effect, may explain why the shrimp larvae survive and thrive in these waters anyway (Tande *et al.*, 1994).

Conclusion

Laboratory experiments may provide significant input information for biological modelling of the shrimp larvae. To ensure the reliability of laboratory results they have to be evaluated against field observations. Tank shape and size should be selected according to the aim of the study. However, density of larvae should not exceed 1 individual 20 ml^{-1}. Natural food in *ad libitum* from the field has to be offered to avoid malnutrition. Lack of pigmentation on the larvae may be used as an early indicator of malnutrition. PCOM is a suitable method for quantitative observations of the behaviour of shrimp larvae in gradients of illumination, salinity, temperature and orientation in relation to food and predators. The PCOM method can be further developed and modified for e.g. observation of fish larvae.

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Table 1. Overview of the laboratory experiments performed on different larval stages of *Pandalus borealis* (→) in progress.

Type of experiment	**ZI**	**ZII**	**ZIII**	**ZIV**	**ZV**	**M**	**Postlarvae**
Temperature dependent growth	x	x	x	x	x	x	→
Observation tanks							
Food-preference	x	x	x	x	x	x	x
PCOM- light	x		x		x		x
PCOM- salinity	x		x		x		x
PCOM- temperature	x		x		x		x
PCOM-predator/prey	x		x		x		x

Table 2. Experimental set-ups used for rearing of *Pandalus borealis* larvae.

No. of larvae in each tank	**No. of tanks in experiment**	**Tank volume**	**Water exchange**	**Water treatment**	**Comments**
1	800	50 ml	Manually, every 48 h	Oxygenation, cooling and Millipore filtered	Good control with moulting. Larvae thrive. Low mortality. All stages. Time consuming (Rasmussen 1993)
50	40	2,5 l	Dripping 2 l h^{-1}	Cooling and Millipore- filtering	Easy to maintain and to observe, the larvae thrive in all stages, especially at the post-larval stages.
300	8	90	8 l h^{-1}	Cooling, sand filtering	Contamination by flexibacta. High mortality, and the larvae had almost no pigmentation. Up to stage ZV.
500	4	250 l	10 l h^{-1}	Sand filtered	Low mortality, easy to maintain, self-cleaning and larvae thrive and all stages present. Removed after settling to 3 l compartments for post-larval studies.

Table 3. Results from the food preference experiments for different stages of *Pandalus borealis* larvae. The numbers indicate the percentage of larvae with gut filling observed every 90 min. up to twelve hours, as average in three trials per food in each stage.

Larvae stage	**Mixed Algae**	**Newly hatched *Artemia sp.***	**Enriched *Artemia sp.***	**Natural plankton Diam.< 125 µm**	**Natural plankton Diam.> 250 µm**	**Artificial food**	**Capelin larvae**	**Cod larvae**
ZI	98	98	89	98	19	100	-	0
ZII	96	94	99	96	21		-	0
ZIII	85	94	94	86	83	83	100 (1999)	6
ZIV	5	94	100	15	96		-	-
ZV	0	91	99	13	94	90	-	-
M	0	99	96	4	100		-	79
Post-larvae	0	80	83		95	100	-	100

Table 4. Example of registration scheme for observation of response of ZI larvae on different illuminations by time (two replicates a and b).

Observation time PCOM-light ZI	0 min a b		30 min a b		60 min a b		90 min a b		120 min a b		Total time
Darkness											
Low illumination											
Medium illumination					1	2			1		5%
Bright illumination			10	10	9	8	10	10	9	10	95%

Table 5. Example of registration scheme for observation of the response of ZI larvae on different salinities (two replicates a and b).

Observation time PCOM-salinity ZI	0 min a	 b	30 min a	 b	60 min a	 b	90 min a	 b	120 min a	 b	Total time
5											
12											
20					1	1					2.5%
25					3	8					14.0%
28					3	1	9	8	10	10	51.0%
30					3		1	2			7.5%
33			10	10							25.0%

Table 6. Example of registration scheme for observation of response of ZI larvae to different temperatures (two replicates a and b).

Observation time TEMP-temp. ZI	0 min a b		30 min a b		60 min a b		90 min a b		120 min a b		Total time
5°C						2	1	2	10		19%
4°C					1	3	2	1		10	21%
3°C				1	3	2	7	7			25%
2°C			2	2	3	3					12%
1°C			8	7	3						22%
0°C											0%

Table 7. List of rearing experiments with *Pandalus borealis* larvae

Authors	**Year**	**Area of origin**	**Aim of study**	**Type of tanks used**	**Food**	**Results**
Berkley	1930	British Colombia	Developmental studies	Tried beakers	Different combinations of food – not specified	Max. ten days survival
Bøhle	1977	North Sea	Growth and development	150 ml glass Petri dishes and transferred to plastic beakers of approx. 600 ml	*Artemia sp.* naupli	7 zoeal stages + Postlarvae stages
Haynes	1979	Gulf of Alaska	Developmental studies	In bottles *in situ*	Naturally occurring plankton	6 zoeal stages
Stickney and Perkins	1981	Gulf of Maine	Developmental studies	3 l beakers and 100 ml compartmental trays	*Artemia sp.* naupli	6 zoeal stages
Wienberg	1982	Helgoland /North Sea	Developmental studies	300 ml glass jars	*Artemia sp.* naupli	9-11 zoeal stages
Nunes	1984	Gulf of Alaska	Temperature dependent growth	600 ml polyethylene beakers	Diatoms, *Artemia sp.* naupli	6-8 zoeal stages
Saotome and Ikeda	1990	Japan Sea	Body composition and metabolism	Various sized tanks 0,5-20 m^3	Diatoms, *Artemia sp.* naupli and fish meat	7 zoeal stages
James	1991		Ontogenetic swimming activity	– not specified	– not specified	6 zoeal stages, settlement at stage ZV
Ouellet and Taggert	1992	Gulf of St. Lawrence	Lipid condition and survival	Rearing tanks, not specified	*Artemia sp.* naupli and *Isochrysis sp.*	Poor TAG conditions and low survival rate in stage ZI-ZIII
Rasmussen	1993 2000	Northern Norway and the Barents Sea	Temperature dependent growth and development	See table 2.	Diatoms, *Artemia sp.* and natural plankton	6 zoeal stages

Figure legends:

Figure 1. *Pandalus borealis* larvae in its first zoeal stage (ZI) newly hatched.

Figure 2. a) 90 l incubators for rearing of zoea stages (ZI-ZIII) of *Pandalus borealis*. Water inlet is in the bottom, outlet on top. In the same picture we see the feeding devices; 5 l PE-bottles with syringes in bottom and thin PE-tubes (ø 1 mm) for food distribution into the incubators.

Figure 2. b) 250 l rearing tanks with automatic cleaning for marine fish larvae used for Z1-M.

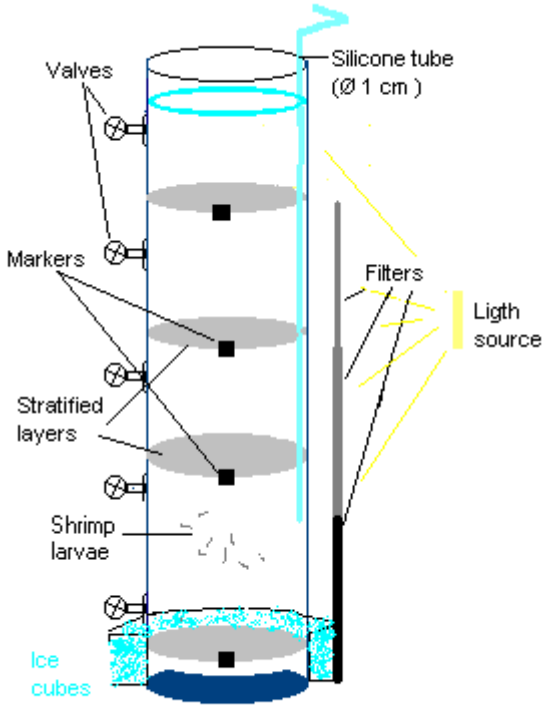
Figure 3. 2,5 l compartment tank set-up for rearing of *Pandalus borealis* larvae (ZI-M).

Figure 4. Abdominal close-up of *Pandalus borealis* ZV with the gut filled with lumps of food (arrows). The separate lumps may present different meals.

Figure 5. The Plexiglas Cylinder Observation Method (PCOM). Ice cubes were only used for temperature preference experiments, light source and filters only for light preference experiments. The silicone tube was used for sampling and adding shrimp larvae and prey organisms into the different layers

a 

b 


Valves
Silicone tube
(Ø 1 cm)
Markers
Filters
Ligth
source
Stratified
layers
Shrimp
larvae
Ice
cubes

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