

Rearing of *Octopus vulgaris* paralarvae: Present status, bottlenecks and trends [☆]

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Abstract

Due to the high mortality rates and poor growth generally observed in *Octopus vulgaris* paralarval rearing experiments, it was decided to organize a working group in order to formulate recommendations to tackle this problem. Over a dozen scientists representing the most active current research groups related to this subject attended the meeting in Vigo, Spain, in November 2005. The aim of this working group was to determine the bottlenecks that prevent success in paralarval rearing, define the most appropriate rearing conditions, and identify required future research.

This paper describes rearing techniques for the *O. vulgaris* paralarvae used by the different research participant teams, with regard to tank systems, feeding environment, and diets (*Artemia*, crustacean zoeae, sandeel flakes, copepods, etc.). Additionally, it includes other related themes such as the culture of *Artemia* and copepods, organisms that are commonly used in paralarval rearing.

When embarking on *O. vulgaris* rearing it is advised to use prey rich in DHA (docosaenoic acid, 22:6n-3) and EPA (eicosapentaenoic acid, 20:5n-3), and with high DHA/EPA ratio. Such prey could be enriched *Artemia*, accompanied or not by crustacean zoeae or any microdiet. It is also recommended that, in future studies, values of growth and survival rates are recorded at the beginning of the benthic phase, in order to compare them to successful previous studies. Dry weight and DHA/EPA ratio of paralarvae may also be good criteria to define paralarval viability and evaluate success of the rearing system.

[☆] This paper is dedicated to the memory of Dr Shigenobu Okumura, who passed away on August 21, 2006.

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It is further concluded that the nutritional aspect is the most important factor influencing larval mortality. Certain lipids (phospholipids, cholesterol, and polyunsaturated fatty acids), amino acids (lysine, leucine and arginine), and essential elements (e.g. copper) play a relevant role in the larval nutrition. It is believed that the PUFA content, especially DHA and EPA, constitutes one of the basic nutritional requirements.

Regarding new research lines, no standardized system for paralarval rearing exists, and it is essential to make progress on this issue. Research on nutritional requirements is considered an area of highest priority, especially the development of a specific enrichment for *Artemia*, the search for alternative live preys, and the development of suitable formulated diets.

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1. Introduction

Octopus vulgaris is one of the best-known and most studied cephalopods in the world. There are compre-

hensive studies on its biology, physiology, behaviour, reproduction, growth, culture, *etc.* (see reviews by: Wells, 1978, 1990; Mangold, 1983; O'Dor and Wells, 1987; Portner et al., 1994; Hanlon and Messenger, 1996;

Vaz-Pires et al., 2004). Therefore, this knowledge constituted an excellent starting point when this species was considered as a new candidate for aquaculture.

The main features of *Octopus vulgaris* subadults and adults that favour its potential for culture are: (1) high food conversion rate, incorporating 40–60% of the ingested food (Mangold and Boletzky, 1973; Wells, 1978; Mangold, 1983); (2) fast growth (around 3% daily growth rate, Mangold and Boletzky, 1973), with males reaching the biggest size (Mangold, 1983; Forsythe and Van Heukelem, 1987); (3) high protein content, representing 70 to 90% dry weight of its body composition (O'Dor and Wells, 1987; Lee, 1994); (4) high fecundity, producing from 100 to 500 thousand eggs per female (Wells, 1978; Mangold, 1983; Iglesias et al., 1996, 1997).

Additional advantages are the social and economic relevance of this species, which is substantiated by its price and the size of its market in the Mediterranean, Latin America and Asia, while at the same time, landings by fishery are declining everywhere (Globefish, 2006). Hence, the culture of octopus from paralarvae to adults constitutes one of the most interesting new species for the aquaculture sector.

O. vulgaris 'paralarvae' (term defined by Young and Harman, 1988) hatch with about 1.0–1.5 mm of mantle length (ML) (ca. 1.5–2.9 mm total size) (Fig. 1). They are planktonic, swim actively and have high metabolic rates, requiring large quantities of live prey of adequate motility and nutritional quality (Villanueva, 1995; Parra et al., 2000; Iglesias et al., 2000; Navarro and Villanueva, 2000, 2003). During the planktonic phase, they undergo strong morphological changes mainly due to the fast growth of the arms relative to the mantle. As a result, the squid-like shape of hatchlings progressively merges to the octopus shape at settlement (Fig. 2). The duration of

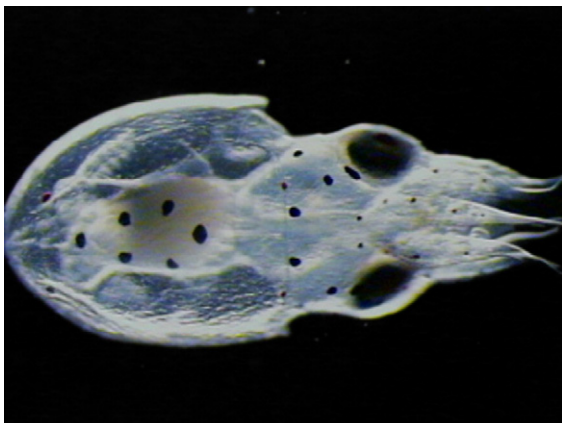


Fig. 1. 0 day-old *O. vulgaris* paralarva (1.5 mm DML, 3 suckers per arm).



Fig. 2. 52 day-old *O. vulgaris* paralarva (5.75 mm DML, 21 suckers per arm).

the planktonic period is temperature-dependent and lasts around 47–54 days at 21.2 °C and 30–35 days at 23 °C (Imamura, 1990; Villanueva, 1995), after which the octopuses start settling to the bottom.

In Japan, Itami et al. (1963) were the pioneers in culturing this species and succeeded to rear the paralarvae using *Palaemon serrifer* zoeae as their main prey. They obtained benthic juveniles at 33 days maintaining a mean temperature of 24.7 °C and reaching a survival rate of 5% at 60 days. Imamura (1990) reported on the main *O. vulgaris* paralarvae rearing experiments carried out in Japan attempting to obtain juveniles for enhancement programs in the 80's. In one of these trials, survival values of 67.1% at day 22 were reached at mean temperatures of 25.1 °C, using *Artemia* as the only prey. Hamazaki et al. (1991) attained 23,700 individuals (28.9% of survival) at day 25 in a 20 m³ tank at mean temperature of 26.9 °C, using only *Artemia* enriched with *Nannochloropsis* sp. as live prey for paralarvae diet. This clearly demonstrates the potential and feasibility of *O. vulgaris* mass production. Nowadays, research on *O. vulgaris* in Japan is basically focused on obtaining benthic juveniles for restocking programs (S. Okumura, personal communication).

It was also during the 90's that Spanish scientists initiated research on *O. vulgaris* aquaculture, focusing both on paralarval rearing and the on-growing of subadult individuals captured from the wild. Villanueva (1994, 1995) and Villanueva et al. (1995, 1996), were able to obtain benthic juveniles of this species from the Atlantic–Mediterranean area with a survival of 9% and 0.8% at 60 days using various crustacean zoeae as a prey and maintaining a mean temperature of 21.2 °C.

Moxica et al. (2002), using *Artemia* and *Maja brachydactyla* zoeae, achieved 8.3% of survival for

one month old paralarvae. In 2001, using the same prey types, the complete life cycle of *O. vulgaris* was closed for the first time (Iglesias et al., 2004), with paralarval survival of 31.5% at day 40 and mean temperature of 22.5 °C. Later, Carrasco et al. (2005) obtained similar results, utilizing the same live preys.

Despite the research effort taken until now, the rearing of *O. vulgaris* paralarvae is generally characterised by high mortality rates and poor growth, which hamper the entire culture of this species.

Due to the difficulty of producing benthic juveniles at a commercial scale, one alternative for the production of *Octopus* was assessed through on-growing subadults in tanks on land and in cages at sea (Iglesias et al., 1996, 1997; Rama-Villar et al., 1997).

In the mid 90s, several private enterprises in Spain (particularly in Galicia) started trials on on-growing of octopus in sea cages that were specifically developed for this purpose. The cages had submerged stacks of PVC tubes, which were used as shelters by the animals. In these experiments, wild-captured octopuses were grown for approximately four months until they reached commercial size (Rama-Villar et al., 1997; Rey-Méndez et al., 2003).

Several Spanish research institutes continued working with *O. vulgaris*, focusing their studies on the on-growing phase (Sánchez et al., 1998; Giménez et al., 1999; García et al., 2001, 2003a,b; Otero et al., 2001; Tuñón et al., 2001; Aguado and García, 2002; García and Aguado, 2002; Cerezo and García, 2003; Muñoz, 2003; Rey-Méndez et al., 2003; Rodríguez et al., 2003, 2006; Oltra et al., 2005). Other studies related to breeding behaviour, spawning, and embryonic development of *O. vulgaris* in captivity were carried out by Moxica et al. (2001).

From 2001 to 2004, a Spanish national research plan (JACUMAR PULPO) was developed, focusing on paralarval rearing and on-growing. The final conclusions of this project revealed that the main problem in the cultivation of this species was the high mortality rates and the poor paralarval growth. This was attributed mainly to the lack of standardized culture techniques and nutritional deficiencies in the diet of paralarvae, especially in n-3 highly unsaturated fatty acids (n-3 HUFA).

Therefore, it was decided that the problem of paralarval rearing needed specific attention, and a working group was organized to examine these bottlenecks. The working group comprised active researchers on this field, either specialised directly in paralarval rearing of *O. vulgaris* or in other relevant aspects that could contribute to resolve the problems (production of a suitable food for paralarvae, tank design and their

implications, etc.). The specific objectives of the working group included: (1) to describe and discuss the different paralarval rearing systems used; (2) to evaluate the mortality causes during the first two months of life; and (3) to establish priorities in the research lines to be followed in the paralarval rearing process.

This document does not intend to be a comprehensive bibliographic revision of the current situation of research on *O. vulgaris* culture, as in fact there is a recent work on this subject (Vaz-Pires et al., 2004), but the objective is to make a review based on the data, discussions and conclusions that arose during the workshop.

2. Methodology descriptions

This section presents the different methodologies and the most relevant results obtained by each of the participating teams. All these groups work directly with paralarval rearing of *O. vulgaris*, or deal with related themes such as the culture of *Artemia* or copepods — organisms commonly used in paralarval rearing.

2.1. Paralarval rearing systems

2.1.1. Paralarval rearing using enriched *Artemia* and compound diets. R. Villanueva (CSIC, Barcelona)

During the first experiments, zoeae from two crustacean species (*Liocarcinus depurator* and *Pagurus prideaux*), were used as live food (Villanueva, 1994, 1995; Villanueva et al., 1995, 1996). However, during recent years the aim of the research focus shifted to the use of artificial prey and the determination of the feeding requirements of the paralarvae using comparative analysis of the biochemical profiles of the reared octopus paralarvae, the wild juveniles and both natural and artificial prey, as well as enzymatic and digestive capabilities of the paralarvae. All experiments were carried out on a small-scale, using replicate tanks of 25–50 L and constant 24 h light. Larvae were fed enriched AF *Artemia* nauplii (450 µm in length and densities of 2 to 10, usually 4–6 nauplii mL⁻¹ day⁻¹) (Villanueva et al., 2002, 2004), *Artemia* biomass supplemented with inert diets such as experimental pellets of 250–500 µm (Navarro and Villanueva, 2000), millicapsules obtained by a gelification and coacervation process (Villanueva et al., 2002; Navarro and Villanueva, 2003), and amino acid solutions added directly to the rearing tank (Villanueva et al., 2004). All these experiments displayed low growth and low survival rates and were limited to the first month of culture, however, they offered an approach to the biochemical and nutritional requirements of the

paralarvae. Navarro and Villanueva (2000, 2003) studied the lipid and fatty acid requirements of the paralarvae and natural preys used in successful cultures (crab zoeae, mysis) showing the relatively high requirements for polyunsaturated fatty acids (PUFA), phospholipids and cholesterol. Poor growth and high mortality in paralarval rearing using *Artemia* nauplii or *Artemia* biomass supplemented with dry or encapsulated microdiets is probably caused by a nutritional imbalance in the fatty acid profile (*i.e.* DHA/EPA ratio) produced by the artificial feeding. The amino acids, lysine, leucine and arginine represented half of the total essential amino acid content of the paralarvae and should be considered for the design of artificial diets. The paralarvae seem to be able to incorporate amino acids directly from the seawater and thereby increase their survival, but its effect on growth is unclear (Villanueva et al., 2004). Analyses of the elemental profiles showed the richness in copper of hatchling octopus, that may indicate a particular nutritional requirement for this element. Reared octopus paralarvae fed *Artemia* nauplii, a prey with relatively low copper content (20 times lower than *O. vulgaris* hatchlings and 8 times lower than *M. brachydactyla* zoeae), showed nearly half of the copper content compared to the “natural” profile in octopus hatchlings or wild juveniles, suggesting a dietary effect (Villanueva and Bustamante, 2006).

2.1.2. Paralarvae rearing with *Artemia* and crustacean zoeae. 1. F.J. Sánchez (I.E.O., Vigo)

In 2001, the closing of the completed life cycle of *O. vulgaris* from paralarvae to adult was achieved for the first time under culture conditions, using the following methodology: two thousand recently hatched (day 0) paralarvae were transferred to a 1 m³ PVC tank at a concentration of 2 ind L⁻¹. The tank was circular (130 cm in diameter) with black wall and white bottom. Mean water temperature was 22.5 °C, and salinity 35 ppt. A 24 h light cycle was provided with two 36 W daylight fluorescent tubes, resulting in an intensity of 600–1000 lux at the water surface. Closed water recirculation with central aeration was maintained during the first week. Microalgae (*Chlorella* sp., *Isochrysis galbana* and *Chaetoceros* sp.) were added daily in order to feed the remaining preys in the culture tank, as to keep them in the best nutritional condition. From day 8 on, the water circulation was partially open (10 L min⁻¹) 4 h per day. The tank bottom was cleaned by siphoning every 4 days. Live diet consisted of adult *Artemia* (1–4 mm total length) cultivated at 25 °C during a week with a commercial cereal mix, and enriched for 24 h with *Chlorella* sp. *Artemia* concen-

tration was of 0.05–0.1 ind mL⁻¹. As a complementary diet, spider crab (*M. brachydactyla*) zoeae were added 4 days per week at a concentration of 0.01–0.1 ind mL⁻¹.

Mean dry weight of paralarvae at hatching was 0.34 ± 0.01 mg. During the first week they actively consumed the adult *Artemia* supplied, but when spider crab zoeae were added as a complementary diet, paralarvae showed a clear preference for this prey. At day 40, survival was 31.5%, the paralarvae had 23 suckers per arm, and they started to migrate to the tank bottom, beginning the settlement process. Mean dry weight of paralarvae at day 45 was 9.5 ± 1.9 mg. The good results obtained with the crab zoeae supply lead us to believe that it is essential to continue with the identification of the essential nutritional components of *Maja* zoeae in order to incorporate these in *Artemia*, and to find a feasible and practical method to use these zoeae. For additional information see Iglesias et al. (2002, 2004).

2.1.3. Paralarvae rearing with *Artemia* and crustacean zoeae. 2. J.F. Carrasco. C.E.P. (Asturias)

In 2002 Carrasco et al. (2003, 2005) succeeded in closing the life cycle of *O. vulgaris* under culture conditions. Although the preys used (*Artemia*, *Maja* zoeae) were similar to those used by Iglesias et al. (2004), the methodology differed considerably. For paralarval rearing, a recirculation system with a 150 L capacity was used. It consisted of three white parabolic polyester fibre tanks, with a diameter of 52 cm, a height of 40 cm with a water distribution system fitted in the base of each tank, a 60 L reservoir, a centrifugal pump, and a biological filter. The seawater flow rate was maintained at about 0.7–1.0 L min⁻¹. Density was 25 paralarvae L⁻¹. Mean water temperature was 21.2 °C and mean salinity 34.4 ppt. Rearing tanks were illuminated with light tubes for 12 h day⁻¹ in every trial. Paralarvae were fed twice daily with live, hatched zoeae of *M. brachydactyla*, maintaining a concentration of 0.7–1.0 zoeae mL⁻¹. Three times a week 7–10 day-old hatched *Artemia* enriched with the microalgae *Tetraselmis suecica* were supplied. *Artemia* concentration was 0.3–0.8 individuals mL⁻¹. Frozen shrimp were also supplied from day 40 on.

At hatching, paralarvae showed an approximate mantle length of 2.1 mm, a dry weight of 0.3 mg, and three suckers on each arm. At day 20, survival was 59% in one experiment and approximately 90% in the two others. After 60 days, 23 benthic individuals survived in one of the experiments, with a survival rate of 3.4%, 91.9 ± 15.33 mg mean wet weight, 22.02 ± 3.8 mg dry weight, and 27–30 suckers on each arm. These results were published by Carrasco et al. (2003, 2005, 2006).

2.1.4. Paralarvae rearing with *Artemia* and crustacean zoeae. 3. J. Roo (I.C.C.M., Canary Islands)

An experiment was carried out to study the nutritional effect of different food sources. Newly hatched octopus paralarvae were fed during the first months with three different live prey combinations: enriched *Artemia* metanauplii (56 h) (A1 Selco; INVE Aquaculture, Belgium), *Grapsus grapsus* zoeae supplemented with enriched *Artemia*, and *Plagusia depressa* zoeae supplemented with enriched *Artemia*. Treatments were carried out in triplicates using 100 L cylindrical fibreglass tanks set with up-welling system for water circulation.

Survival rate was significantly improved with the use of both crab zoeae (11–27% with *G. grapsus*; 7–10% with *P. depressa* at 28 days after hatching [dah]) in comparison to diet exclusively based on *Artemia* (0–18.5% at 22 dah). Paralarvae dry weight was also higher in treatments using crabs zoeae (1.14 ± 0.49 mg with *G. grapsus*; 1.12 ± 0.22 mg with *P. depressa* at 28 dah) than in those exclusively fed on *Artemia* (0.78 ± 0.02 mg at 20 dah). Besides, some histological alterations on the digestive gland morphology were observed in paralarvae fed exclusively on *Artemia*. Biochemical composition of preys showed that the beneficial effect of the crabs zoeae supplementation could be related to a higher essential fatty acid (EFA) content, particularly arachidonic acid (ARA) content. Although it was showed that levels of n-3 HUFA, and mainly DHA in the zoeae, seem to be insufficient to meet paralarvae requirements. (Socorro et al., unpublished data).

2.1.5. Paralarvae rearing using enriched *Artemia* and *Ammodytes personatus*. S. Okumura. Y.S. (Japan)

Octopus paralarvae were reared during the first months being fed two types of *Artemia* (large and small), supplemented with frozen flakes of Pacific sandeel (*A. personatus*). Rectangular tanks of 500 and 4000 L were used, with constant illumination and water temperature kept at 25 °C. Survival, growth and HUFA composition were determined in paralarvae and food organisms.

Survival at day 32 was significantly higher with large *Artemia* (45.9%) than with the smaller ones (2.7%). Similarly, the mean wet weight was 37.3 mg for the 32 day old paralarvae fed large *Artemia*, whilst 18.2 mg fed smaller ones.

Supplementing sandeel flakes improved the DHA/EPA ratio of paralarvae, since both types of *Artemia* contained only minimal DHA. DHA/EPA ratio in 32-day-old paralarvae of both *Artemia* groups was equal (1.5), similar to that of hatchlings and wild subadults (Okumura et al., 2005).

2.1.6. Paralarvae rearing using *Artemia* and copepods. E. A. G. Vidal. UFPR. (Brazil)

Due to morphological and behavioural similarities between *Loligo* squid and *Octopus* paralarvae the rearing techniques developed for squid (Vidal et al., 2002, 2005) were applied to *Octopus* paralarvae with the aim of improving survival rates. In the case of *Loligo*, the tank hydrodynamics (small cylindrical tanks, 1.0 m diameter, 220 L, and a circular current, 1.0 to 1.4 cm s⁻¹) contributed to reduce skin and fin damage of paralarvae and thus, improved their survival.

Therefore *O. vulgaris* paralarvae were reared in a closed recirculation system including six replicate tanks of 100 L with attached filtration (see Vidal et al., 2002 for details) at 20 ± 1 °C and fed a diet of enriched *Artemia* nauplii supplemented with copepods (*Acartia tonsa*), juvenile mysids (*Metamysidopsis elongata atlantica*) and crab zoeae (*Callinectes sapidus*). Food was supplied 4 times a day and prey densities maintained above 0.15 prey mL⁻¹. Mean dry weight of paralarvae at hatching was 0.24 ± 0.2 mg and growth rates ranged from 4.1 to 4.8 dry weight day⁻¹ at day 40. Survival rates varied greatly from 1 to 39% at day 40, and the best survival was obtained with the utilization of the copepod *A. tonsa* up to day 15 as a supplement to enriched *Artemia*.

It was observed that *O. vulgaris* paralarvae were being subject to mortality caused by damage in their arms and mantle. The damages were caused by constant friction against the tank walls and bottom. Initial damage in the arms was noticed from 5 to 10 day after hatching and the ventral arms were the most affected due to constant contact with the bottom of the tanks. The degree of damage was closely related to the small size of the tanks used and to the hydrodynamic behaviour and related circulation (circular current) inside the same. Therefore, different hydrodynamic configurations and directions were tested and the best results (*i.e.* small incidence of arms damage) were obtained with a gentle ascending fluid motion that reduced the contact of paralarvae with the tank and bottom walls. These results clearly show that tank design and fluid motion are key factors for paralarval survival, as it has been observed for fish (Rasmussen and McLean, 2004) and need further investigations.

2.2. Prey production methods

2.2.1. Intensive cultivation of the copepod *A. tonsa* and its utilization in *O. vulgaris* larviculture. J. G. F. Bersano FURG (Brazil) and E. A. G. Vidal. UFPR (Brazil)

Interest on the mass cultivation of copepods as an alternative live food for aquaculture has increased since the early 1990s, partly because of the global shortage of

Artemia (Støttrup and McEvoy, 2003). Marine copepods have a higher nutritional value than rotifers and *Artemia*, (Watanabe et al., 1983; Sargent et al., 1997; Støttrup and Nosker, 1997), making them an attractive live food source to be used in the larviculture of fish larvae and invertebrates. Nevertheless, the use of copepods is still limited in aquaculture, due to the lack of a standard cultivation method and in most cases, the difficulty of getting large numbers of individuals in a short period of time. In the last years, an intensive method to culture copepods has been used in southern Brazil, with relative success (Bersano, 2003). It consists of a cylinder-conical 500 L black fibreglass tank, fitted with an airlift system and an egg collector that allows the collection of eggs every 24 h.

This method was developed for the calanoid copepod *A. tonsa*, a planktonic free-spawning species, but other calanoid species can be also considered. The food used for the copepods consists of a mixed algal diet, made up of *Thalassiosira fluviatilis* and *I. galbana*. The stocking density is kept about 50 females L⁻¹ and 30 males L⁻¹ and checked every seven days. Tank production can be assessed every 24 h by eggs and nauplii counting. Maximum production is approximately 1.2–1.4 million nauplii day⁻¹ per tank, with a mean production per culture volume about 1600–1800 nauplii L⁻¹ day⁻¹. Copepods produced with this method are currently being used in a series of experiments that include the paralarval rearing of *O. vulgaris*. In these experiments adult individuals of *A. tonsa* (~1 mm) were used in a stocking density of around 80 copepods L⁻¹ as a supplement to enriched *Artemia* nauplii during the first 15 days of *O. vulgaris* larviculture. The first results obtained are promising with survival rates from 20 to 38% at day 40 after hatching, and specific growth rates of around 4.6% dry weight day⁻¹.

2.2.2. Copepods production and biochemical composition. T. van der Meeren. I.M.R. (Norway)

Mesocosms (large enclosed bodies of water) can be manipulated to enhance zooplankton production (mainly copepods) (Naas et al., 1991). This has been regularly done in a 20,000 m³ seawater pond at IMR-Austevoll, Norway, between 1984 and 2001. The most important factors for managing the production are fertilisation to enhance primary production, mixing to prevent anoxic layers, and salinity control. A complete mesocosm draining is also needed to prevent the settlement of competing or predatory organisms on copepods. Copepods can be harvested by means of plankton filters (e.g. UNIK-900 wheel-filter) (van der Meeren, 1991; van der Meeren and Naas, 1997). There is a large production

potential, and an average of ca. 80 million copepods has been collected daily during a period of 4–5 weeks at IMR-Austevoll.

Biochemical components of copepods from the mesocosm, including lipid classes, fatty acids of total lipid, free amino acids, total protein, protein-bound amino acids, pigments (astaxanthin and β -carotene), vitamins (A, B₁, B₂, C, D₃ and E), have been analysed from April to December over two years (van der Meeren et al., 2001; van der Meeren, 2003). Copepod size, dry matter content, and fraction of ash were also determined. Results show that copepods were surprisingly stable regarding several nutritional components. Copepods may be characterised by moderate lipid levels (10–15% dry weight), dominated by polar lipids (approximately 60% total lipid). Three fatty acids were most abundant in total lipid, 16:0 (ca. 15%), EPA (ca. 15%), and DHA (ca. 30%). ARA fraction was very low (less than 1%), which gives an EPA/ARA ratio of about 24, while the DHA/EPA ratio was around 2. The free amino acids fraction (FAA) was between 5 and 8% copepod dry weight, and a correlation between the FAA amount and salinity was found. FAA were dominated by glycine, arginine and taurine based on concentration. On average, 19 and 24% of FAA concentration were essential FAA. Protein determined from protein-bound amino acids (PAA) was 41 and 44% of copepod dry weight. Essential amino acids fraction in PAA was approximately 40% of amino acid concentration. Glycine, glutamine/glutamic acid, alanine, asparagine/aspartic acid, and leucine were the most abundant PAA. Copepods were very abundant in astaxanthin (600–700 μ g/g dry weight), while β -carotene was not found. High concentrations of vitamin C (400–500 μ g/g dry weight) and vitamin E (ca. 110 μ g/g dry weight) were found, while vitamin A and D₃ occurred in trace amounts or were not detected.

Despite that nutritional requirements for octopus paralarvae are not yet completely defined, mass production of copepods in mesocosm may provide a transitional method for obtaining sufficient paralarval feed of reasonable nutritional quality, both for future research and for commercial exploration of methodology.

2.2.3. Use of ongrown *Artemia*. J. Dhont. Ghent University (Belgium)

Artemia is the most commonly used live food organism in commercial aquaculture but the vast majority uses freshly hatched nauplii or additionally enriched nauplii of 24 h old. However, in particular cases the use of larger *Artemia* is beneficial due to size and nutritional differences. Iglesias et al. (2006) affirm

that *O. vulgaris* paralarvae prefer adult *Artemia* to nauplii. Offering larger prey leads to a higher food intake per hunting effort, substantial saving of cysts and reduction of costs. The nutritional value of adult *Artemia* is determined by the phytoplankton species or other food sources used during its culture, and eventually the nutritional quality of *Artemia* can easily be improved via enrichment products once the desired size is reached.

The intensive culture of *Artemia* requires appropriate culture water, an *Artemia* strain that is easily available, consistent in quality and has proper growth characteristics, and a suitable diet. A suitable diet includes live algae, dry algae, yeasts and ground agriculture by-products (soy pellets, rice bran, wheat, etc.). The drastic impact of the fortuitous microflora is responsible for huge variations in the culture success when using yeasts or powdered feeds; a proper recirculation infrastructure is also needed to preserve the water quality.

Cultures can be run in a stagnant system, with open or closed flow through (and every intermediate) (Dhont and Lavens, 1996). Stagnant (=batch) systems require a simple infrastructure and daily maintenance, but water quality quickly deteriorates and does not allow for cultures of more than one week at moderate animal densities (about 5000 animals L⁻¹). For longer cultures some kind of water treatment becomes necessary. Treatment may consist of a physical treatment to remove particles that are too big for *Artemia* to ingest (sedimentation tank, plate separator, protein skimmer, etc.) and/or a biological treatment to maintain nitrogen compounds and bacterial populations below acceptable levels (rotating biodisc, sandfilter, trickling filter, etc.). Culture results can be diverse, but using phytoplankton

it is possible to get *Artemia* with a size of 2 mm in 4 days, 4 mm in a week and up to 8 mm in two weeks with a survival not lower than 70%. More detailed information can be found in Dhont et al. (1991) and Dhont and van Stappen (2003).

3. Comparison of broodstock handling and paralarval rearing techniques

Once the various currently used paralarval culture systems and their culture performance were presented, a comparison was made, including reproduction with the aim to unify parameters, standardize the existing methodology and prepare recommendations. This information is shown in Tables 1 and 2. The most marked differences in relation to broodstock handling (Table 1) were male:female ratio, which is not a very determining factor, and the wide range of temperatures utilized (14–25 °C). Capture methods, transport conditions, food used and light were similar.

Table 2 shows that the biggest differences among the paralarvae rearing methods were tank volume, water system, light intensity and photoperiod, temperature and paralarvae and prey density. In contrast, common aspects concerning tank shape, aeration, and the use of crustacean zoeae and enriched *Artemia* were reported.

4. Recommendations

Based on the description of the various methods used for octopus rearing, and on ensuing discussion in the working group, a series of recommendations that could be useful for those researchers that take their first steps in the

Table 1
Summary of *O. vulgaris* broodstock capture methodology, transport and maintenance used by the different research groups

	Barcelona	Vigo	Canary Island	Asturias	Andalusia	Brazil	Japan
	ICM-CSIC	IEO	ICCM	CEP	IFAPA	FURG	YS
Capture method	Traps	Traps	Traps	Traps	Traps and hooks	Pots and diving	Pots, hooks and lines
Transport	Oxygen supply Low density	Oxygen supply Low density	Oxygen supply	<15 min no water >15 min aeration	Aeration	Oxygen supply, aeration	Oxygen supply
Sex ratio (male:female)	1:5–8	1:2	1:3	1:2–3	1:1	1:2–3	No males
Food	Crab, fish	Crab, fish, mussel	Crab, fish	Crab, fish	Crab, fish	Crab, fish	Crab, mantis shrimp, fish
Temperature (°C)	Reduce to induce spawning 14–23	Reduce to induce spawning 15–20	18–22	15–20	15–22	19–23	15–25
Light	Darkness	Dim	Shaded	Dim	Shaded	Dim	Shaded (90% reduction)

Table 2
Summary of paralarval rearing conditions of *O. vulgaris* carried out by different research groups

Parameters	Barcelona ICM-CSIC	Vigo IEO	Canary Islands ICCM	Asturias CEP	Andalusia IFAPA	Brazil FURG	Japan YS
Tank volume (L)	25–50	1000	100	30	400	100	500
Tank colour	Black	Black	Grey	White	Black, grey	Black	Orange
Tank shape	Cylindrical and parabolic	Cylindrical	Cylindrical	Parabolic	Cylindrical and rectangular	Cylindrical	Cylindrical
Water system	Open	1st week stagnant, 2nd semi-open (3–4 h = 100%)	Open 25% day ⁻¹	Open (recirculation)	Open	Closed (recirculation)	5 first days stagnant then open system
Aeration	No	Yes, intermediate	Yes, gentle	Yes, gentle (cleaning)	Yes, gentle	No	Yes, gentle
Light	24 h bulb 60 w 900 lx at 1 cm under the water surface	24 h fluorescent (2) 36 W 2000 lx	Natural photoperiod	12 h light–12 h darkness; fluorescent (1) 40 W	Natural photoperiod	10 h light–14 h darkness; natural + cold light 19–24	Fluorescent (1) 36 W
Temperature	19–23	20–22	21.5–22.5	20–22	19–22	19–24	25
Clear/green water	Clear	Green <i>Isochrysis</i> + <i>Nannochloropsis</i>	Clear	Clear	Green <i>Tetraselmis</i> + <i>Isochrysis</i>	Clear	Freshwater <i>Chlorella</i>
Paralarvae density (ind. L ⁻¹)	13–48	5	25	25	20	5–30	3
Type and prey density (ind. mL ⁻¹)	Zoae <i>Liocarcinus</i> and <i>Pagurus</i> nauplii <i>Artemia</i> (2–6) and <i>Artemia</i> biomass	Zoae <i>Maja</i> (0.01–0.1) (when available) + <i>Artemia</i> (0.05–0.1)	Zoae <i>Grapsus</i> (15 ind. L ⁻¹) + <i>Artemia</i> (72 h) (2)	Zoae <i>Maja</i> (0.7–1) + <i>Artemia</i> (3 times/week) (0.5–0.7)	Zoae (<i>Carcinus</i> , <i>Palaemon</i> and <i>Maja</i>) (<0.1) + <i>Artemia</i> + <i>Moina</i> (4–5 day-old) (1.0)	Crustacean zoae, copepods, mysids, nauplii and adult <i>Artemia</i> 0.15–0.3 (4–5 takes)	<i>Artemia</i> nauplii + fish flakes from 5th day
Size of prey	Zoae (1.3–3.1 mm TL) <i>Artemia</i> nauplii to 1–3 mm <i>Artemia</i> biomass	Zoae: 1 mm TL <i>Artemia</i> : 2–3 mm TL	Zoae: 1.5 mm TL <i>Artemia</i> : 0.85 mm TL	Zoae: 1 mm TL <i>Artemia</i> retained in 300 µm sieve	Zoae: 0.8–1.0 mm <i>Moina</i> : 1.0–1.2 mm <i>Artemia</i> : 1–3 mm	0.4–8 mm	650 µm (<i>Artemia</i>) 1–2 cm diameter, 0.5–1 mm thickness (FF)
<i>Artemia</i> enrichment	DCSuperSelco, Metionine	Reared in commercial cereal flour, enriched with <i>Nannochloropsis</i> (5.10 ⁶ cell mL ⁻¹)	<i>Artemia</i> enrichment (A ₁ Selco Inve)	Reared and enriched with <i>Tetraselmis</i>	Reared and enriched with <i>Tetraselmis</i> + <i>Isochrysis</i> SuperSelco, Prolon	Super SELCO and DHA SELCO Inve	Fish egg powder (Plus Aquaran, BASF Japan)
Sampling	Every 7–10 days	Every 7 days	Every 7 days	Every 10 days	Every 7–10 days	Daily up to day 7 and every 5 days thereafter	Every 5 days
Survival	0.8% at day 60 with zoae, and 54% at day 20 with <i>Artemia</i> nauplii (with poor growth)	31.5% at day 40	11–27% at day 30	89.6–93.5% at day 20 and 3.4% at day 60	5–15% at day 35	1–20% at day 40 with <i>Artemia</i> and from 20–39% at day 40 with <i>Artemia</i> and copepods	10–30% at day 30
Cleaning	Daily tank bottom siphoning	No bottom cleaning until day 30	No bottom cleaning	Every 20 days changing tank by pipetting and checking the survival	Daily tank bottom siphoning	Bottom siphoning daily or every other day	Daily tank bottom siphoning after 5th day

study of paralarval rearing of *O. vulgaris* was prepared. These recommendations are summarised in the following sections.

4.1. Reproduction

Nowadays, each laboratory successfully obtains eggs and viable hatchlings in sufficient amounts to develop paralarval rearing trials. Nevertheless, it is possible to give some specific recommendations to improve and standardize methods to obtain paralarvae for rearing:

To collect subadult and adult individuals from the wild, the use of basket traps is strongly recommended in order to avoid damage during captures of broodstock individuals. In addition, this gear is very selective and reduces fishery impact on the environment.

Mortality of adults during transport can be prevented through aeration, in the case of low densities, or through an oxygen supply for high densities. The use of individual PVC pipes or net bags could also reduce mortality.

In order to enhance the biochemical composition of paralarvae, it is recommended to include at least 30% of crustaceans in the broodstock diet. Low commercial value crustaceans and fish are specifically suitable.

If individuals are captured during the spawning season, it is sufficient to obtain mated females (> 1 kg), otherwise, during the rest of the year, males and females can be kept at a ratio of 1:3, respectively.

To avoid disturbance among individuals, spawning females and their respective egg masses may be transferred to separate tanks from the broodstock.

Handling and transport of breeding females and/or egg masses should be done before the second embryonic inversion to prevent premature hatching. Furthermore, sudden changes in the physical environment (*e.g.* temperature, salinity, pH, light intensity), as well as mechanical stimuli, should be avoided as much as possible.

Care must be taken when collecting and transferring hatchlings in order to avoid stress and damages. Their skin is extremely delicate and can be easily damaged. Sampling after hatching must be carried out gently, either through individual counting or volumetric estimation.

4.2. Paralarval rearing

Due to the lack of knowledge on the basic biological, nutritional, physical, and behavioural requirements of octopus paralarvae, considerable and widely different efforts have been made to explore the feasibility of rearing these molluscs. Therefore, a wide variety of tank forms and colours, light conditions, as well as water supply, quality and turbidity has been tested. Further-

more, a number of prey types and sizes have been applied, often under different feeding regimens (concentrations, feeding times, and food treatments). As a result, no standardized methods exist at the present for paralarval rearing. However, information provided in Table 2 by the working group leads to the following recommendations:

In case small volume tanks are used for the paralarval rearing trials, a water circulation system that avoids skin damages in paralarvae, especially in the arms, will be required.

When using larger tanks (more than 500 L) an appropriate distribution of paralarvae and their preys in the water column should be sought. This can be achieved through the circulation system (water intake and outlet) or by gentle aeration.

Enriched *Artemia* are recommended as live prey due to its easy manipulation and availability, together with copepods, crustaceans zoeae or a suitable microdiet.

Regarding the lipid composition of reared paralarvae and that of their natural food, it can be deduced that they require a food source rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol, and a moderate content in neutral lipids. Any diet used (*Artemia* enriched by any method, a microdiet or any live prey) should also present a high DHA/EPA index.

Additionally, any experiment on rearing paralarvae should provide data on growth in dry weight and survival rate when the benthic phase is reached (approximately 2 months of age at 21 °C). The values published in this review on the successful experiences that used *Artemia* as a prey supplemented with crustaceans zoeae can be used as a reference.

DHA/EPA ratio and dry weight of the paralarvae, related to temperature at a certain age, could be good criteria to define the paralarval viability and to evaluate the success of the rearing system.

5. Discussion

Acclimatisation of breeders in captivity does not pose any problem in octopus culture. The main recommendations to be taken into account to achieve good acclimatisation and spawning have been quoted above. Some authors put males and females together (Iglesias et al., 2000; Roo, personal communication), and others separate individually fertilised females caught at sea (Villanueva, 1995; Okumura et al., 2005). Most females mature and spawn in captivity, and a high hatching rate is frequently obtained.

One of the key issues that was discussed by the working group was the cause for the high mortality recorded during the planktonic phase of the octopus life

cycle. Two possible factors initially mentioned were the lack of a standardised paralarval rearing system, and the absence of an appropriate food supply that meets the nutritional requirements of paralarvae. It became evident that even using two different culture systems (tank volume, colour, preys and paralarvae density, water flow, etc.) similar growth and survival results were obtained after supplying the same type of diet (*Artemia* and *Maja* zoeae) (Iglesias et al., 2004; Carrasco et al., 2005). This points out to nutrition as a key factor in octopus paralarval rearing success.

It is generally agreed that *Artemia*, specifically the *franciscana* strain, constitutes an attractive and well-accepted prey for octopus paralarvae. Nevertheless, there is much discussion about the ideal size that should be used. Some authors (Vidal et al., 2002; Navarro and Villanueva, 2003) use *Artemia* nauplii (approximately 450 µm and 750 µm after enrichment) during the first weeks of culture, due to the easy production and lipid enrichment of this prey. Other authors (Moxica et al., 2002; Iglesias et al., 2004; Carrasco et al., 2005) used *Artemia* of ca. 2 mm of total length even in the beginning of the rearing process, and they consider that this size of prey could be suitable until the first month of culture; the results attained by Hamazaki et al. (1991), who used *Artemia* (1.5–2 mm) enriched with *Nannochloropsis* sp., support this trend since they obtain survival of 28.9% on day 25 (until the onset of the benthic phase).

From the first month onwards, paralarvae need a prey larger than *Artemia* or crustacean zoeae. Although experiments with large prey like the mysid *M. elongata atlantica* (E. A. G. Vidal, unpublished data) and the amphipod *Talitrus saltator* have been carried out (Carrasco et al., 2003), the results are not conclusive yet.

Even though *Artemia* is well accepted and produces positive results until one month after hatching (especially if it is enriched), *Artemia per se* shows a biochemical composition that does not meet the nutritional requirements of octopus paralarvae, lacking long chain PUFA, essential for marine animals, like EPA which may be present in low amounts or absent in the lipid of the brine shrimp, and DHA which is basically absent (Navarro et al., 1992, 1993). This fact was also demonstrated in experiments carried out exclusively using enriched *Artemia* as live diet for paralarvae, where very low growth and survival indexes were recorded (Iglesias et al., 1997; Villanueva et al., 2002). The biochemical composition of the produced paralarvae is notably different than that of paralarvae reared with crustaceans zoeae namely by its lipid content that is especially lower PUFA (Navarro and Villanueva, 2000; Moxica et al., 2002). The only experiments using *Ar-*

temia as the single diet and producing high paralarval survival during the first month after hatching, have been those experiments utilizing the microalgae *Nannochloropsis* sp. as enrichment for *Artemia* (Hamazaki et al., 1991; Moxica et al., 2006). Okumura et al. (2005) also attained good survival results at the end of the planktonic phase using *Artemia* and sandeel flakes, pointing out the importance of the DHA.

The first experiment using the copepod *A. tonsa* in combination with enriched *Artemia* (Vidal, methodological descriptions section) showed encouraging results when compared to a single diet of *Artemia*. This was probably related to the high nutritional quality of copepods, naturally rich in essential highly unsaturated fatty acids (HUFA), phospholipids and natural antioxidants (Sargent et al., 1997).

Several other research groups have reached the benthic phase (Itami et al., 1963; Villanueva, 1995; Iglesias et al., 2002, 2004; Carrasco et al., 2003, 2005) with very different culture conditions and systems. They had in common the use of live shrimp or crab zoeae (*P. serrifer*, *M. brachydactyla*, and *P. prideaux*) alone, or as a complement food together with *Artemia*. The biochemical composition of these crustaceans is characterised by a high phospholipid content, lack of triglycerides and high percentage of PUFA (n-3), specifically DHA (Navarro and Villanueva, 2000) which may be very important during early development due the high demand for membrane synthesis in rapidly growing larvae (Henderson and Sargent, 1985). These characteristics seem to explain the lipid requirements of octopus, and suggest shrimp and crab zoeas to be very valuable during the first period of paralarval feeding on live feed. Consequently, a diet made up of enriched *Artemia* and shrimps or selected crabs zoeae is currently the most appropriate to achieve better survival and growth indexes to reach the benthic phase.

This methodology enabled the completion of the octopus life cycle under culture conditions, and yielded juveniles that reached the adult stage and spawned in captivity (Iglesias et al., 2004; Carrasco et al., 2005). Nevertheless, this method cannot be applied on a commercial scale, as the availability of live zoeae is limited and its culture difficult to scale up to commercial levels. For these reasons it is considered essential to continue the search for a live prey or an *Artemia* enrichment that covers more efficiently the basic nutritional requirements of paralarvae.

In fact, much progress has been achieved in this regard. The importance of DHA and EPA fatty acids in paralarval diet has been evidenced. Protein and lipid biochemical profiles of paralarvae and their preys —

highly important to determine this species' nutritional requirements during its planktonic phase (Navarro and Villanueva, 2000, 2003) — have been achieved. Future studies based on joint feeding (*Artemia* and microdiets) should consider a feed balanced in phospholipids, cholesterol and polyunsaturated fatty acids (specially DHA), with proteins rich in lysine, leucine and arginine (Navarro and Villanueva, 2000, 2003; Villanueva et al., 2002, 2004), and rich in essential elements as copper (Villanueva and Bustamante, 2006). Obtaining a balanced microdiet with an appropriate palatability and acceptance by the paralarvae is also an important objective to be reached.

Further investigation is needed on the reasons of the high acceptability of crab zoeae by octopus paralarvae and the better growth recorded when this prey is used as a food supplement. The interesting lipid profile of copepods and frozen flakes of sandeel (*Ammodytes* sp.) will equally yield interesting DHA values justifying the continuation of research on this topic (Okumura et al., 2005; F. Linares, personal communication).

Regarding tank design and other more appropriate physical conditions for rearing octopus paralarvae, no standardized model could be suggested, due to the fact that, as shown in Table 2, each team uses different volumes (from 25 to 9000 L), colours (from black to white, moss green or grey), photoperiod (0, 12 or 24 h) or circulation systems (open, closed or semi-open). There is a general agreement on the fact that the main factor to be taken into account in selecting a proper culture tanks is that it allows homogeneous distribution of both paralarvae and prey in the water column. In order to achieve this aim, some authors (Iglesias et al., 2004; Okumura et al., 2005) use a gentle aeration at the bottom of the tank produced by diffuser stones, avoiding the formation of small bubbles that could get trapped inside the paralarvae mantle. Others use special water distribution and diffusion systems, such as a dripping water diffuser (Villanueva, 1995) or multiple superficial and tangential water inlets (Vidal et al., 2002), and even others (Carrasco et al., 2005, 2006) use a water inlet at the bottom of the tank, producing a vertical and upwards circulation, similar to that used for lobster culture. The combination of tank design and volume, aeration and position and flow of the water inlet can produce a large array of configurations and hydrodynamics to which paralarvae were exposed to during rearing. Therefore, future studies that evaluate the impact of the rearing environment on the survival of paralarvae are much needed.

Additionally, standardization of the feeding process (prey density, doses, etc.) is a shortcoming still to be solved in future research.

6. Future research lines

6.1. Reproduction

Although this phase is generally under control there are some aspects subject to improvement.

6.1.1. Control of the sexual maturation

To understand the effects of environmental stimuli that control maturation, more research is needed to obtain spawns in any season. This includes investigation on physical factors like temperature, photoperiod and salinity, as well as biotic factors like food availability, mating behaviour and the endocrine system.

6.1.2. Genetic selection

Genetic selection is needed to improve essential features of octopus culturing. This may include characteristics such as growth, maturation, and immune competence.

6.1.3. Paralarval quality

Research on the effects of broodstock conditions (size, nutrition, etc.) and environmental factors (temperature, light, etc.) are necessary to ensure the supply of large numbers of high quality paralarvae.

More defined criteria to determinate paralarval quality (related to biochemical composition, yolk content, hatching weight, etc.) need to be established.

6.1.4. Pathology

Investigation on pathology related to broodstock and egg masses is needed.

6.2. Paralarval rearing

6.2.1. Feeding requirements

Paralarval nutrition is a major source of variation. Research on nutritional requirements, prey acceptability and nutrient absorption should be carried out. This includes exploration of new live preys, biochemical composition of the diets, paralarval energetic demands, and development of formulated diets. The working group considered this area of research to be of the highest priority.

Other research lines to be considered are:

6.2.2. Tank system and turbulence

Research is needed to improve knowledge on the effects of system and tank design and fluid motion on paralarval survival and growth (e.g. the effects of tank design, water inlet, aeration, and water outlet on tank hydrodynamics).

6.2.3. Water quality

Suitable water quality parameters should be determined to ensure optimal paralarval rearing and reduction of variability (e.g. factors like microbial population, use of probiotics, recirculation, open circuit system, gas supersaturation, and tolerance limits to, for example, metabolites like carbon dioxide and ammonia).

6.2.4. Feeding environment

Establishment of optimal feeding environment should be explored to ensure that paralarvae needs are satisfied (e.g. the effect of tank colour, turbidity, green water/clear water, light intensity and quality, photoperiod, prey density, prey size, and feeding schedule).

6.2.5. Paralarval behaviour

Paralarval responses to different biological and physical stimuli need to be mapped and quantified (e.g. predator–prey interactions, phototaxis, and chemotaxis).

6.2.6. Paralarval development

Research on aspects related to paralarval morphology, physiology, growth dynamics, and internal rhythms, needs to be carried out, aiming at understanding proper fitness of the paralarvae along the planktonic development.

6.2.7. Pathology

Identification of specific pathogens and bacterial growth in the rearing systems should be also taken into account.

7. Conclusions

It was concluded that the nutritional aspect is the most important factor of influence on paralarval mortality. Phospholipids, fatty acids and cholesterol among lipids; lysine, leucine and arginine, among amino acids, and copper among the essential elements, play a relevant role in the larval rearing process. It is believed that the PUFA content, especially DHA and EPA, constitutes one of the basic nutritional requirements.

The best results of paralarvae rearing have been achieved using diets of high PUFA level (Itami et al., 1963; Villanueva, 1995; Iglesias et al., 2002, 2004; Carrasco et al., 2003, 2005; Okumura et al., 2005); satisfactory results at around one month culture were also attained using *Artemia* enriched with *Nannochloropsis* sp.

However, in order to achieve conclusive results, live shrimp or selected crab zoeae seem to be the most appropriate prey to use as control.

More basic research is needed on nutritional requirements (lipids, proteins, essential elements, vitamins), physiology, behaviour and pathology. Furthermore, in

order to enable for comparisons between research groups and to determine the success of the tested parameter it is essential to include survival and growth at initial benthic phase in future studies.

Zootechnical research is also needed on tank (type and design, colour, volume, etc.), water (quality, circulation, turbulence), and exploration of new live prey types (copepods, mysids, shrimps, etc.).

Finally, the working group members recognised the importance of a close coordination for further research between the different laboratories, and are committed to the internal exchange of information on new developments in order to maximize progress and minimise overlapping efforts.

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