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Drinking activity and microparticle size selection in early post-hatching axenic European sea bass (*Dicentrarchus labrax* L.) larvae

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A B S T R A C T

Marine fish larvae are known to drink water and to feed selectively. We studied water and microparticle ingestion of axenic sea bass larvae at the early post-hatching stage. Knowledge on these physiological processes is crucial for designing effective feeding, particle delivery schemes for marine fish larvae and for the understanding of the interaction and/or association between larval fish and smaller microparticles such as bacteria in aquaculture systems. We found that the fluorescence intensity of accumulated FITC-labelled dextran in the gut of laboratory-reared axenic European sea bass larvae (*Dicentrarchus labrax*), at day after hatching 7 (DAH7), correlated to a drinking rate of 4.1 ± 0.1 nL h^{-1} $larva^{-1}$. Additionally, by providing microparticles of 2, 10 or 45 μm, each time in combination with reference microparticles of 20 μm at an equal particle volume, feed size selection was examined. Feeding bigger particles (a combination of 45 and 20 μm) resulted in a statistically higher mean ingested volume ($10^5 \pm 10^4$ $μm^3$ $larva^{-1}$) than feeding particle combinations of 2 and 20 μm or 10 and 20 μm, ($10^4 \pm 10^3$ $μm^3$ $larva^{-1}$). Based on Jacobs' selectivity index (*D*-values), fish larvae always selected for the bigger particles. In addition, larvae showed significantly positive selection towards 45 μm particles after 12 h of feeding. We conclude that axenic European sea bass larvae at DAH7 fed selectively on larger microparticles and unintentionally ingested small microparticles through drinking. Thus, both active and unintentional uptake of microparticles can be useful for microparticle delivery in early post-hatching marine fish larviculture. These results produce insight into the possibility of feeding with an appropriate particle size for future studies using early post-hatching axenic European sea bass larvae as model animal.
Statement of relevance: Axenic European sea bass larvae exhibited drinking activity at DAH7 and our data from the particle size selection are relevant for the use in developing of an effective feeding and microparticles delivery schemes in sea bass larviculture. In addition, unintentional uptake of small sized particles could be useful in the application of beneficial probiotic bacteria through suspension feeding.
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1. Introduction

Both marine and fresh water fish are known to drink water in order to adapt to their environment. Marine fish are hypotonic to their environment because their internal osmotic concentration is lower than of the surrounding water. Therefore, their water loss via osmosis is being compensated by drinking sea water (Charmantier et al. 2005). Vast amount of information of osmoregulation in adult marine fish is available (Evans 2008). However, knowledge on drinking activity by marine fish larvae is still limited to only several species such as cod, plaice, turbot and European sea bass (Mangor-Jensen and Adoff 1987; Reitan et al. 1998; Tytler and Blaxter 1988; Varsamos et al. 2004). Nevertheless, no information is available on the drinking activity in axenic European sea bass larvae. So far, axenic sea bass larvae have been used to study host microbial interactions (Dierckens et al. 2009; Li et al. 2014; Rekecki et al. 2012).

The drinking activity in fish larvae is closely associated with the ingestion of particles that are present in the water column. This ingestion of particles is part of the fish feeding behavior, which is influenced by several factors such as the availability of appropriate prey (e.g. size and density), the fish ability to capture, handle and ingest the food. Knowledge on the larva's feeding behavior and factors that modulate food detection, capture and ingestion by fish larvae are vital for

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developing feeding strategies. Selection of the correct particle size is important at the feeding onset for European sea bass larvae in order to comply with the size of larval mouth opening and to ensure optimal ingestion of feed. The opening of the mouth of sea bass larvae is situated between DAH4 and DAH5. At that moment, the mouth size is ranging between 170 and 320 μm (Silva 2007). Previously, microparticulated compound diet ranging from 60 to 120 μm was used to feed conventional European sea bass larvae at DAH6 to 13, while microparticulated compound diet ranging from 120 to 200 μm and 200 to 400 μm were used from DAH14 to 25 and from DAH26 onwards, respectively (Cahu and Zambonino 1994). The use of fluorescent latex (polystyrene) inert microparticles have been proven useful to aid in the visualization of the ingestion of particles by zebra fish (Cocchiaro and Rawls 2013; Field et al. 2009) as well as in filter-feeding organisms like *Artemia* and polycheata (Fang-Yen et al. 2009; Makridis and Vadstein 1999).

The objectives of this study were to gain further insight into drinking activity and microparticles size selection in axenic European sea bass larvae. To determine the drinking activity, we have opted to use an inert tracer because of its non-hazardous nature and hence its ease of handling. While particles size selection was studied by feeding sea bass larvae with inert fluorescent latex (polystyrene) microparticles preventing digestion and thus facilitating the exact quantification of ingested particles. Investigation of size selection using axenic sea bass larvae could potentially avoid interference with naturally occurring microparticles such as microorganisms. To our knowledge, the present study is the first to measure the drinking rate, ingested volume and particle size selection using inert particles in early post-hatching axenic European sea bass larvae.

2. Material and methods

2.1. Axenic sea bass larvae

Naturally spawned European sea bass (*D. labrax*) eggs were obtained from Ecloserie Marine (Gravelines, France). Hatching of axenic sea bass larvae was done according to the protocol described by Dierckens et al., (2009). Unless otherwise stated, larvae were stocked in filtered (0.45 μm; Sartobran P, Sartorius Stedim Belgium N.V., Vilvoorde, Belgium) autoclaved artificial sea water (Instant Ocean, United Pet Group, Virginia, US), at a salinity of 36 g L^{-1} and were kept in a temperature-controlled room (16 ± 1 °C) with dim light (10 cd steradian m^{-2}). A rifampicin solution was added together with filtered autoclaved artificial sea water at a final concentration of 10 mg L^{-1} into the stocking vials. Vials of stocked larvae were placed on a rotor turning at 4 rpm with an axis tangential to the axis of the vials to provide aeration and avoid sedimentation. Larvae were unfed in all experiments to avoid interference with the determination of the drinking rate and particle uptake. Axenity was tested by adding 30 eggs and 1 mL of culture medium from each incubation bottle to 9 mL of sterile marine broth (Carl Roth GmbH Co., Karlsruhe, Germany) followed by incubation at 28 °C for 96 h. Incubation bottles that were positive for growth of bacteria or fungi were not included in the experiment. This experiment was carried out in accordance with the recommendations in the European Union Ethical Guidelines for the care of animals used for experimental and other scientific purposes (2010/63/EU).

2.2. Measurement of the drinking rate

At DAH6, five biological replicates of 34 larvae were collected and stocked one by one in a sterile transparent screw cap vial in 9 mL filtered autoclaved artificial sea water. Rifampicin was not added to avoid fluorescence intensity interference. At DAH7, the drinking rate was determined by adding 1 mL fluorescein isothiocyanate (FITC) labelled dextran (MW 4000 Da; Sigma Aldrich, Diegem, Belgium) to each vial at a concentration of 1 mg mL^{-1} (10 mL final volume per vial). Larvae in vials added with 1 mL filtered autoclaved artificial sea water without FITC-labelled dextran served as a control. Ten larvae per replicate were sampled at each time point; 20, 40 and 60 min after addition of FITC-labelled dextran, anaesthetized (0.1% benzocaine) and washed three times with filtered autoclaved artificial sea water. The fluorescence background of individual larvae was very similar. Hence, ten larvae were placed in a flat bottom 96-well microplate with five replicates and fluorescence intensity was determined using a fluorescent microplate reader (Tecan Infinite M200, Mechelen, Belgium) at 490 nm excitation with 520 nm emission. The drinking rate was determined by comparing the intensity of accumulated fluorescence in the gut using a standard curve derived from fluorescence intensity of serially diluted FITC-dextran (1 mg mL^{-1}) in filtered autoclaved artificial sea water containing larvae homogenate in triplicate. Establishment of the correlation is possible due to the transparency of the tegument lining of the sea bass larvae at DAH7. Correlation coefficient was determined by linear regression equation relating the volume of fluorescent dextran (x, nL) and fluorescent intensity (I_f) in the gut of larvae at the time of exposure (min), $I_f = 252.444x + 257.68$, $R^2 = 0.99$.

2.3. Microparticles

Fluoresbrite® polystyrene latex microparticles (Polyscience Inc., Pennsylvania, USA) were counted using a Bürker haemocytometer (LO Laboroptik Ltd., Lancing, UK) and Sedgewick-Rafter counting chamber (Fisher Scientific, Aalst, Belgium). Particles from each size were suspended in sea water at an equal volume of 2.0×10^9 μm^3 mL^{-1} in order to avoid confounding bead size with total offered bead volume. All microparticles were yellow green fluorescent with 441 nm excitation and 486 nm emission spectra. Particles with three different sizes were tested (2, 10 and 45 μm) against particles of 20 μm (median size) as the reference size. Particle uptake was always compared between two bead sizes (one of three test sizes and the reference size) to increase the accuracy of the estimated selection index (Baer et al. 2008).

2.4. Measurement of the ingested volume and size selection

At DAH6, 12 larvae were stocked in 9 mL filtered autoclaved artificial sea water in three biological replicate. At DAH7, three different combinations (2 and 20 μm; 10 and 20 μm; 45 and 20) and a control with only 20 μm of Fluoresbrite® microparticles were added into the vials equally for a total volume of 4.0×10^9 μm^3 mL^{-1}. The number and volume of ingested microparticles were determined by sampling the larvae after 2, 4, 8 and 12 h of feeding. Larvae were anaesthetized with 0.1% benzocaine and rinsed gently three times with sterile filtered artificial sea water on 100 μm mesh sieve to remove microparticles that were uneaten or sticking to the body. Subsequently, larvae were transferred into glass vials, vigorously vortexed with 1 mL of 65% nitric acid (HNO_3) followed by addition of 4 mL of distilled water. Finally, 1 mL of 30% sodium hydroxide (NaOH) was added to neutralize the pH of the samples. Dissolved larvae were filtered on 0.2 μm Whatman™ Black Polycarbonate Membranes (Sigma Aldrich, Diegem, Belgium) and the number of microparticles was counted using a solid phase cytometer (ChemScan C; Chemunex, Ivry-sur-Seine, France) and epifluorescence microscopy (Olympus BX40; Olympus Belgium N.V., Aartselaar, Belgium).

In addition, determination of size selection was done using a modified version (Baer et al. 2008) of Jacobs' selectivity index (Jacobs 1974). Jacobs' selectivity index (D) was calculated for two microparticle sizes, A (test size) and B (reference size), as:

$$D_A = (r_A - p_A)/(r_A + p_A - 2r_A p_A) \quad (1)$$

where

$$r_A = f_A/(f_A + f_B) \text{ and } p_A = N_A/(N_A + N_B) \quad (2)$$

r_A fraction of latex microparticles of size A in the guts of the larvae
p_A fraction of latex microparticles of size A in the medium

f_A number of latex microparticles of size A in the guts of the larvae
f_B number of latex microparticles of size B in the guts of the larvae
N_A number of latex microparticles of size A in the medium
N_B number of latex microparticles of size B in the medium
D_A selectivity for A ranges from -1 to $+1$, where $D = 0$ indicate unselective feeding, $D = +1$ indicate positive selection and $D = -1$ indicate negative selection for microparticles of size A.

2.5. Statistics

Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). Data used for parametric analysis of variance (ANOVA) were tested for homogeneity of variance (f-test, $p < 0.05$). One-way ANOVA between groups was used to compare differences of the drinking rate, total ingested volume of microparticles (combination of test and reference size) and the percentage of total ingested volume of test particles at $p < 0.05$. ANOVA tests were followed by post-hoc comparisons using Tukey HSD test. Independent-samples t-test ($p < 0.05$) was used to compare Jacobs' selectivity index D-values against a D-value of zero.

3. Results

3.1. Measurement of the drinking rate

Axenic sea bass larvae at DAH7 showed a mean ($\pm$ SD) drinking rate of 4.1 ± 0.1 nL h^{-1} larva^{-1} after being exposed to FITC-labelled dextran. There were no statistically significant differences in drinking rate between the three time points (20, 40 and 60 min), indicating a constant drinking rate for at least 1 h.

3.2. Measurement of total ingested volume

Larvae at DAH7 were fed with a combination of test (2, 10 and 45 μm) and reference (20 μm) microparticles. Ingested volumes were measured after 2, 4, 8 and 12 h of feeding and the mean ingested volumes for these four time points were calculated. Feeding a combination of 45 and 20 μm particles always resulted in a statistically higher mean ingested volume ($10^5 \pm 10^4$ μm^3 larva^{-1}) than feeding particle combinations of 2 and 20 μm or 10 and 20 μm. For the latter two, the mean ingested volume at the four examined time points was identical ($10^4 \pm 10^3$ μm^3 larva^{-1}) (Fig. 1). Larvae fed a combination of 45 and 20 μm particles always ingested a significantly higher volume at each time point than larvae fed with particle combinations of 2 and 20, 10 and 20 μm or only 20 μm.

Regardless the examined time point, larvae always preferred the largest particles within each particle combination used (Fig. 2). Thus, particles of 45 or 20 μm were preferred above particles of 2 and 10 μm. For the particle combination of 45 and 20 μm, at each time point larvae had eaten a high percentage of 45 μm particles, e.g. at 12 h, 95 $\pm$ 0.5% of the ingested particles were 45 μm. While for the other two combinations, at 12 h, larvae had eaten 20 $\pm$ 8% of the 10 μm particles and only 0.10 $\pm$ 0.07% of the 2 μm particles.

3.3. Measurement of size selectivity

As proposed by Jacobs (1974), selective feeding occurs when a feeder consumes co-occurring feed at a different rate. The selectivity index (D) can range from $+1$, over 0 to -1 indicating a positive selection, no selection and negative selection, respectively. At 2, 4 and 12 h, sea bass larvae always exhibited a positive selection towards bigger size particles (Fig. 3). Additionally, a significantly positive selection towards 45 μm size particles was observed at 12 h.

4. Discussion

4.1. Sea bass drinking rate

Marine fish are known to drink water to compensate for osmotic losses. Here we measured the drinking rate of axenic sea bass larvae at DAH7. To our knowledge, there are only 4 similar studies, examining the drinking rate in marine fish larvae at DAH7, DAH2, at mouth opening or the yolk sac stage and juvenile (Mangor-Jensen and Adoff 1987; Reitan et al. 1998; Tytler and Blaxter 1988; Varsamos et al. 2004) (Table 1).

In our study, sea bass larvae at DAH7 (3.5 mm larval standard length or 0.5 mg mean wet body weight) that were kept for 1 h at a salinity of 36 g L^{-1} and water temperature of 16 °C showed a constant drinking rate of 4.1 ± 0.1 nL h^{-1} larva^{-1}, which is lower than the drinking rate of European sea bass larvae obtained in a previous study (Varsamos et al. 2004). In spite of that, a direct comparison of the drinking rate as such is rather difficult, due to the difference in units used in the study of Varsamos et al. (2004). Also, in that study a different salinity was used (5 and 25 g L^{-1}) relative to the one used in our study (36 g L^{-1}). This salinity was used based on the axenic sea bass model developed

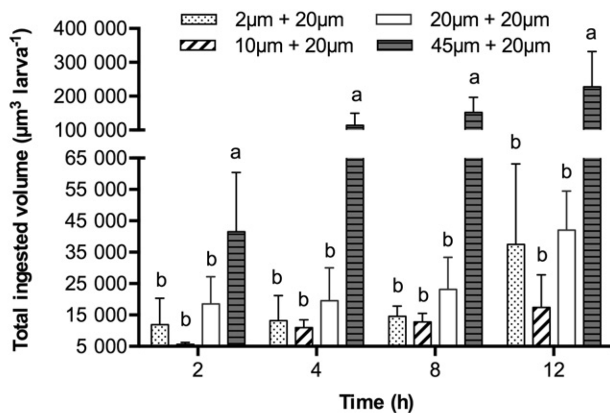

Fig. 1. Total ingested volume for each test size and 20 μm reference particles at different time points. Larvae fed with a combination of smaller microparticles (2 and 20 μm or 10 μm and 20 μm) showed a ten times reduction in the total ingested volume of microparticles, 10^4 μm^3 larva^{-1}. The total ingested volume of 2 μm particles represents mainly the reference particles (20 μm) as very few 2 μm particles were detected. Vertical bars represent standard deviation ($n = 3$). Letters denote significant differences in total ingested volume of the microparticles between different size combinations at each time point, (One-way ANOVA, Tukey HSD, $p < 0.05$).

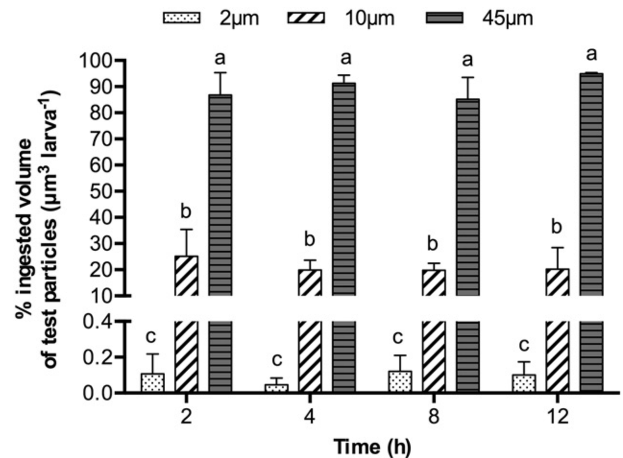

Fig. 2. Ingested volume of test microparticles expressed as percentage per larva compared to the total ingested volume of reference microparticles. Particles of 2 μm were the least ingested compared to the particles of 10 and 45 μm, and represented by a very small percentage between 0.05 and 0.10% after 12 h of feeding. Vertical bars represent standard deviation ($n = 3$). Letters denote significant differences of percentage ingested microparticles between test sizes (One-way ANOVA, Tukey HSD, $p < 0.05$).

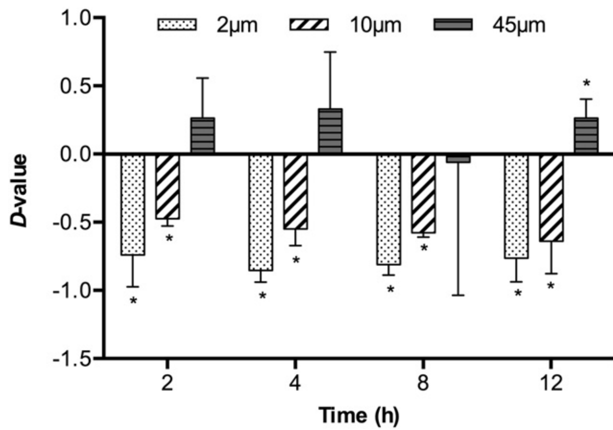

Fig. 3. Selectivity index (*D*-values) of fish larvae fed on test and reference particles. Positive values indicate that larvae fed two different sizes (test and reference particles) always selected the bigger particles. Additionally, larvae showed a significantly positive selection of 45 μm particles after 12 h of feeding. Negative selection was observed for the small sized microparticles of 2 and 10 μm against reference particles at all time points. Vertical bars represent standard deviation ($n = 3$). Asterisks denote significant differences between calculated *D*-values and a *D*-value of zero (*t*-Test, $p < 0.05$).

in our laboratory (Dierckens et al. 2009). In older larvae, juvenile and adult fish, it is known that the drinking activity is influenced by water salinity (Skadhauge and Lotan 1974; Varsamos et al. 2004). However, a comparison of the drinking rate (normalized to body weight) in yolk sack stage of European sea bass larvae showed no significant difference at different salinities (Table 1) (Varsamos et al. 2004). This suggests that the low drinking rate by European sea bass larvae in our study was not the consequence of the high salinity used. In early post hatching, fish larvae adaptation to changes in water salinity is controlled through osmotic permeability of the integument or skin surfaces. It is equipped with goblet cells for gas exchange and ionocytes or chloride cells for ion exchange (Varsamos et al. 2004; Schreiber 2001) which showed the ability to expand cell size as the salinity increases (Hiroi et al. 1999). European sea bass larvae are shown to be able to osmoregulate through tegumentary ionocytes (Giffard-Mena et al. 2006).

The low drinking activity in our axenic sea bass perhaps depends on the accuracy of the measurement techniques. In most studies on drinking rate, assessment was done using inert isotope tracers, especially ^{3}H-labelled dextran. Here we used FITC-labelled dextran, which is a safer alternative inert marker compared to radioisotope tracer. Earlier, the use of a radioactive tracer and FITC-labelled dextran was compared in larvae of three different marine fish species (Tytler and Blaxter 1988) (Table 1). DAH32 herring larvae and DAH4 plaice larvae at a salinity of 32 g L^{-1} showed, depending on the technique used, a similar difference in drinking rate. When measured with the ^{3}H-labelled dextran-based method, drinking rate yields slightly lower values compared to FITC-dextran-based method. So, the rather low FITC-dextran drinking rate obtained in this study does not seem to be inherent to that methodology. Although the data are consistent with the general claim that marine fish larvae drink water, there is no information about drinking activity in axenic marine fish larvae. Hence, we speculate that the complete absence of particulate compounds could have an influence on the drinking rate in European sea bass larvae and this should be a topic for further research. If this hypothesis would be substantiated, it would mean that drinking rate values should only be reported if particle concentration is measured simultaneously.

4.2. Sea bass microparticles ingestion

Currently, we showed that axenic sea bass larvae were able to ingest inert latex particles at first feeding, if being offered at appropriate size. Larvae showed 100% survival up to 12 h allowing us to assume that the size of microparticles at 2, 10, 20 and 45 μm were not interfering with the ingestion (data not shown). Independent from time and the particle size combination, larvae always preferred the largest particles (Fig. 3). Thus, particles of 45 or 20 μm were preferred above particles of 10 and 2 μm. Previously, sea bream larvae (*Sparus aurata*) with a total length <4 mm preferentially ingested particles of 25 to 50 μm and larvae of 4 and 5 mm preferred particles of 51 to 100 μm (Fernández-Diaz et al. 1994). Additionally, ingestion of the smallest inert microparticles (2 μm) in our study showed that the unintentional uptake of the particles occurred through drinking activity. Similar observations have been made in yolk sac larvae of cod which have been shown to ingest bacterial particles before exogenous feeding (Hansen and Olafsen 1999; Olafsen 2001). Unattached bacteria could also be ingested by tilapia fry (*Oreochromis niloticus*) after a bacterial suspension was introduced to the culture water (Beveridge et al. 1989). Consequently, our information related to the unintentional uptake of smaller

Table 1
Comparison of drinking rate in other marine fish species.

Species	Salinity (g L^{-1})	Temperature (°C)	Age (DAH)	Drinking rate (^{3}H-labelled dextran) (nL h^{-1} larva^{-1})	Drinking rate (FITC-labelled dextran) (nL h^{-1} larva^{-1})	Drinking rate (^{51}Cr-EDTA) (nL h^{-1} mg^{-1})	Author(s)
Gadus morhua (cod)	34	5	7	3	–	–	Mangor-Jensen and Adoff (1987)
Clupea harengus (herring)	32	7.5	35	46.6	[a]65.1	–	Tytler and Blaxter (1988)
	16	7.5	35	19.2	[a]35.9	–	
Pleuronectes platessa (plaice)	32	7.5	16	24.1	[b]27.0	–	
	16	7.5	16	16.6	[b]35.0	–	
G. morhua (cod)	32	7	4	7.5	–o	–	
	32	7	19	26.8	18.7	–	
	16	7	19	10.3	–		
Scophthalmus maximus (turbot)	34	12 to 18	2 to 11	14.0 to 120.0	–	–	Reitan et al. (1998)
D. labrax (European sea bass)	5	17	Mouth opening (0.55 ± 0.1 mg)	–	–	[c]59.9	Varsamos et al. (2004)
	25	17		–	–	[c]63.9	
D. labrax (European sea bass)	36	16	7	–	4.1	–	This study

[a] Drinking rate was based on larvae of day after hatching 41 (DAH41).
[b] Drinking rate was based on larvae of day after hatching 4 (DAH4).
[c] Drinking rate was normalized and expressed per wet weight of the larva.

particles could be useful for aquaculture, such as in the application of beneficial probiotic bacteria through suspension feeding.

First feeding of marine fish larvae such as in the European sea bass (*D. labrax*) (Barnabe 1976), gilthead sea bream (*S. aurata*) (Yúfera et al. 1999) and Dover sole (*Solea solea*) (Appelbaum 1985) can be done using artificial or microencapsulated diet. However, the size of these diets is important to ensure uptake and ingestion of the diet by marine fish larvae. Previously, a study in Asian sea bass (*Lates calcarifer*) between DAH3 to DAH14, feeding larvae all-protein-membrane microcapsules with a size range from 15 to 150 μm, demonstrated ingestion in the range of 40 to 60 μm (Walford et al. 1990). The intestinal transit of the smaller particles posed no problems. In contrast, larger particles (90 to 150 μm) showed an extended intestinal passage time and their passage through the intestinal-rectal valve was extremely difficult. We also noticed that combination of larger particle sizes (45 and 20 μm) was causing obstruction in the gut that may have caused mortality when fed for >12 h (data not shown).

4.3. Size selectivity

Marine fish larvae are generally considered as a visual feeders at the beginning of trophic life as the eyes become pigmented at the mouth opening stage of the larvae (Yúfera and Darias 2007). Thus, feeding axenic sea bass larvae with particle sizes that are appropriate relative to the developmental stage at early post-hatching, i.e. mouth opening is important to ensure that the fish larvae could visualise, capture and ingest the offered particles. The success in capturing a feed particle by fish larvae, after their sensory organs localised a particle is also being determined by an appropriate ratio of the larval mouth gape and the particle size. In this case, with the known mouth size of sea bass ranging between 170 and 320 μm (Silva 2007), ingestion of the given inert microparticles was shown to be possible.

At the early exogenous feeding, ingestion of feed particles by fish larvae is mainly by swallowing due to the lack of capability to bite (Boglione et al. 2003; Kohno et al. 1983). The prey is also selected by its size more than taste or other factors (Yúfera and Darias 2007). It has been shown that fish larvae can ingest prey with similar size to their mouth gape, but usually prefer smaller prey with prey/gape ratio of 25–50% (Yúfera and Darias 2007). The range of accessible prey or particle size increase as the fish larva grows. In this study, we observed a selective feeding behavior in early post-hatching axenic sea bass larvae. The size selection was being determined using only a combination of two particle sizes rather than a mixture of many particle sizes to increase the accuracy of the estimated selection index (Baer et al. 2008). Furthermore, the use of inert microparticles can also avoid the interference of other factors that might affect size selection such as particles' surface characteristics, feeding attractants and nutritional quality (Baer et al. 2008). Jacobs' selectivity index (D) indicated a positive selection for large (45 μm) and negative selection for small particles (2 and 10 μm) compared to the reference size of 20 μm. Thus, feeding fish larvae with a larger particle size ensured an active uptake of the particles into the gut lumen. Ingestion of the 2 and 10 μm particles could be due to passive or unintentional uptake through drinking activity. This unintentional uptake of smaller microparticles through drinking is suggested to continue even when the eyes are well developed.

5. Conclusions

Our study addressed a short-term inert microparticle feeding of laboratory-reared axenic European sea bass larvae at DAH7. Larvae exhibited drinking activity and preferably consumed inert microparticles of bigger size within each particle combination used. Particles of 45 μm were preferentially selected above particles of 20, 10 and 2 μm. Ingestion of smaller particle sizes happened rather unintentionally through drinking. In conclusion, an appropriate particle size selection allows microparticle delivery at early post-hatching in marine fish larvae through unintentional and active uptake of microparticles.

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