University of Crete
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Department of Biology

Sea bream (*Sparus aurata*) larvae foraging behavior under different rearing mediums

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Ευχαριστίες

Η παρούσα εργασία εκπονήθηκε στα πλαίσια του προγράμματος (BIOEXPLORE, 09ΣΥΝ-23-941) και της «Επιτροπής ερευνών» του Πανεπιστημίου Κρήτης, Ελλάδα. Αριθμός προγράμματος 2809/ 05-2009 – 12-2013.

«Ευχαριστώ πολύ Κυρία Κεντούρη…» θα πω απλά. Μετά από σχεδόν δύο χρόνια, που βρίσκομαι στο εργαστήριο της συμπεριφοράς υπό την επίβλεψή σας, ακόμα δεν μπορώ να πιστέψω πόσο τυχερή έχω σταθεί. Δεν ξέρω αν είναι δυνατό ένας φοιτητής να είναι τόσο τυχερός όσο εγώ. Από την πρώτη στιγμή με δεχτήκατε στο εργαστήριο σας χωρίς κανένα δισταγμό, αν και θα μπορούσατε να έχετε πολλούς… Με εμπιστευτήκατε και με στηρίξατε έμπρακτα και το κάνετε ακόμα. Η συνεργασία και η επαφή μαζί σας είναι για μένα μια εμπειρία που θα μου μείνει αξέχαστη καθώς μόνο θετικά και ευχάριστα έχω να θυμάμαι. Η εκτίμηση μου στο πρόσωπό σας δε σταματά στο ερευνητικό/επιστημονικό επίπεδο. Σας εκτιμώ ακόμα περισσότερο ως άνθρωπο κι αυτό για μένα είναι το πιο σημαντικό. Σας ευχαριστώ και πάλι από καρδιάς, για όλα όσα μου δώσατε τη δυνατότητα να ζήσω, να μάθω, να …καταφέρω κοντά σας!

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…Αχ Ελλάδα, Σ'αγαπώ και βαθιά σε ευχαριστώ...

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Περίληψη

Οι νυμφικές καλλιέργειες αποτελούν ένα από τα πλέον ευαίσθητα και ευάλωτα στάδια στην παραγωγική διαδικασία των ιχθύων. Συνεπώς, βέλτιστες συνθήκες εκτροφής αποτελούν κρίσιμο σημείο καμπής στην υδατοκαλλιέργεια, αναδεικνύοντας την ανάγκη να ερευνηθούν οι παράγοντες που επηρεάζουν την επιβίωση και την ανάπτυξη των νυμφών. Ως άμεσο αποτέλεσμα αυτής της προσπάθειας είναι η αύξηση της παραγωγής και η προσφορά καλύτερης ποιότητας του τελικού προϊόντος.

Έχει φανεί, ότι οι συνθήκες εκτροφής, όπως η θερμοκρασία, η ιχθυοφόρτιση, η παροχή τροφής, η ένταση του φωτός, η θολερότητα του περιβάλλοντος νερού και η χρήση «πράσινου νερού» ως μέσο εκτροφής επηρεάζουν άμεσα την επιβίωση και την ανάπτυξη των νυμφών των ψαριών. Ειδικότερα, η χρήση μικροφυκών ως μέσο εκτροφής στις νυμφικές καλλιέργειες αποτελεί σήμερα κοινή πρακτική καθώς επιδρά θετικά στην αύξηση, στην ανάπτυξη και στην επιβίωση των νυμφών. Οι πληροφορίες που υπάρχουν όμως, σχετικά με την επιρροή των μικροφυκών στη συμπεριφορά θήρευσης της ζωντανής τροφής των νυμφών, είναι ελάχιστες. Για το λόγο αυτό, πρέπει να ερευνηθεί αν και πώς επηρεάζει η θολερότητα του μέσου εκτροφής (παρουσία μικροφυκών ή άλλων ουσιών) τη θηρευτική συμπεριφορά των νυμφών συγκριτικά με τη χρήση καθαρού νερού (απουσία θολερότητας) που εφαρμόζεται επίσης σε διάφορα είδη.

Στην πλαίσια της παρούσας εργασία έγινε καταγραφή του ρυθμού αύξησής των νυμφών και αναλύθηκε και ποσοτικοποιήθηκε η θηρευτική τους συμπεριφορά σε τρία διαφορετικά μέσα εκτροφής. Παράλληλα, καταγράφηκε η διαμόρφωση του προτύπου θήρευσης της ζωντανής τροφής κατά τα πρώτα αναπτυξιακά στάδια των νυμφών. Πραγματοποιήθηκαν, λοιπόν, δύο πειράματα σε πλήρως ελεγχόμενες εργαστηριακές συνθήκες. Για κάθε ένα από τα δύο πειράματα χρησιμοποιήθηκαν 50000 αυγά τσιπούρας τα οποία ισοκατανεμήθηκαν σε εννέα πειραματικές δεξαμενές. Στο 1^{ο} πείραμα ελέγχθηκαν, σε τριπλέτες, τρία μέσα εκτροφής, το φυτοπλαγκτόν που αποτελούνταν από το μικροφύκος, *Chlorella minutissima,* τα διάτομα που αποτελούνταν από διάλυμα σκόνης διατόμων και το καθαρό νερό. Στο 2^{ο}

πείραμα το φυτοπλαγκτόν και το καθαρό νερό παρέμειναν, ενώ τα διάτομα αντικαταστάθηκαν από τη χλωροφύλλη (διάλυμα σκόνης χλωροφύλλης).

Η καταγραφή της δραστηριότητας των νυμφών στις δεξαμενές έγινε με τη χρήση καμερών, που ήταν τοποθετημένες στο πάνω μέρος των δεξαμενών. Οι καταγραφές διαρκούσαν μια ώρα και γίνονταν δύο φορές τη μέρα για το $1^{ο}$ και τρεις φόρες ημερησίως για το $2^{ο}$ πείραμα. Η παροχή τροφής στις νύμφες γινόταν στο μέσο της περιόδου καταγραφής. Στις νύμφες παρέχονταν ζωντανή τροφή που περιελάμβανε αρχικά Τροχόζωα (*Brachionus plicatilis*) ενώ στη συνέχεια προστέθηκαν και καρκινοειδή (*Artemia sp.* nauplii). Κατ' εξαίρεση, στο $2^{ο}$ πείραμα δόθηκε στις νύμφες και βιομηχανική τροφή κατά τις τελευταίες μέρες του πειράματος. Επίσης, πραγματοποιήθηκαν και εβδομαδιαίες μετρήσεις που αφορούσαν στην παρακολούθηση και στον έλεγχο της ποιότητας του νερού των δεξαμενών για τη διασφάλιση σταθερών συνθηκών στην καλλιέργεια ($2^{ο}$ πείραμα).

Από τη συλλογή των αποτελεσμάτων προκύπτει ότι υπάρχει διαφοροποίηση στο ρυθμό αύξησης μεταξύ των τριών μέσων εκτροφής (σε κάθε πείραμα). Η συμπεριφορά θήρευσης μεταβάλλεται σε συνάρτηση με τα αναπτυξιακά στάδια των νυμφών τσιπούρας. Τέλος, ο αριθμός των επιθέσεων στη ζωντανή τροφή σχετίζεται με το μέσω εκτροφής.

ABSTRACT

Larviculture still represents the most important stage on fish production. Larvae survival and growth are correlated with the rearing parameters, such as temperature, stocking density, food supply, light intensity, water turbidity, use of green water, etc. Particularly, the use of microalgae in the rearing medium is a common procedure, nowadays, since it positively influences the survival, growth and development of most farmed fish larvae. However, quite scarce information exists regarding the potential effect of microalgae on larval foraging behavior. In addition, whether turbidity created by microalgae or equivalent water turbidity opposed to clear water medium affect feed intake during early developmental stages of fish is yet to be explored.

We investigated: a) Quantification and analysis of larval foraging behavior under three different rearing mediums b) larvae foraging pattern towards live feed along the developmental stages, and c) the growth rate. Two successive experiments were conducted under fully-controlled laboratory conditions. A total number of 10^5 sea bream eggs were used in this study, equally distributed to nine experimental tanks (5000eggs/tank/experiment). In the 1st experiment, three rearing mediums were tested in triplicates: Phytoplankton Medium (PM), consisting of *Chlorella minutissima*, Diatom Medium (DM), consisting of diatom's powder solution and Clear water Medium (CM). In the 2nd experiment, DM was replaced by Chlorophyl Medium (ChM), which consisting of chlorophyll powder solution.

Larvae activity in tanks was being recorded twice every day in the 1st and three times per day in the 2nd experiment. Recordings were focused on the feeding time, where live feed (*Brachionus plicatilis* (Trochozoa) and *Artemia sp.* nauplii (Crustacean) were provided. Exceptionally in the second experiment, small amount of moisture was provided. Further, profile measurements regarding the water quality in tanks was performed every week to ensure stable conditions for larviculture.

From the results obtained, it can be concluded that a) the foraging behavior alters in relation with the developmental stages of sea bream larvae,

b) the number of attacks was associated with the rearing medium and c) growth rate varies within the three rearing mediums (in each experiment).

INTRODUCTION

Definition of aquaculture

Aquaculture is the farming of aquatic organisms such as: fish, mollusks, crustaceans, aquatic plants, crocodiles, alligators, turtles, and amphibians. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. It also implies individual or corporate ownership of the stock being cultivated (FAO, 2008)

Aquaculture production

Aquaculture is the most rapidly growing animal food-producing sector. The contribution of aquaculture to global supplies of fish, crustaceans, mollusks and other aquatic animals has significantly grown over the past half-century (figure 1).

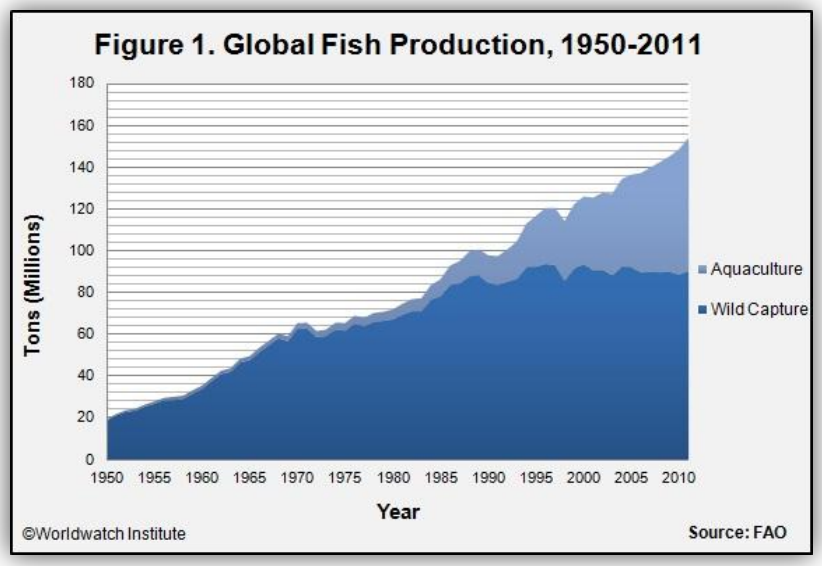

Figure 1: The diagram shows the development of the Global fish production from 1950 to 2011. Aquaculture is growing rapidly having reached 40% of the total production. (FAO, 2010)

Mediterranean aquaculture

The first records of aquaculture in the Mediterranean region can be traced back to Ancient Egypt at 2500 BC, where tilapia farm existed on small ponds. Later, Etrucsans (Italy) had marine fish farms while Greeks cultivated shellfish

in the 5th century BC. Romans used to breed marine fish, in particular sea bass and seabream, which were considered very valuable.

Modern Mediterranean aquaculture started in the ‘80s, mainly dedicated to sea bass and sea bream farming. Today, the sector produces over 300.000 tons versus a few thousand tons 20 years ago. Although sea bass and sea bream make up for 95% of total production, new species (meagre, dentex, red porgy, sharpsnout seabream etc.) have been gradually introduced in the industrial domain.

Greece is the main producer for sea bass and sea bream, maintaining a stable share of over 40% of the world production. Turkey follows with 30%, while the remaining 30% is produced in other Mediterranean countries.

The Mediterranean fish farming sector presents a significant growth that resulted to a remarkable production of domestic fresh, cheap and high quality fish. In addition, it contributes to a socio-economic structure, particularly in the fisheries-dependent areas, while provides productive activity to uninhabited islands and rock-islands which are normally excluded from other investments.

Sparus aurata

Etymology

Sparus aurata: Latin, sparus & aurata (=golden) = a golden fish.

Taxonomy

- Kingdom: Animalia
- Phylum: Chordata
- Subphylum: Vertebrata
- Superclass: Osteichthyes
- Class: Actinopterygii
- Order: Perciformes
- Family: Sparidae
- Genus: Sparus
- Species: *Sparus aurata,* (Linnaeus, 1758)

Figure 2: Sea bream individuals in natural habitat (http://www.magrama.gob.es/gl/pesca/temas/proteccion-recursos-pesqueros/reservas-marinas-de-espana/cabo-de-palos-islas-hormigas/galeria-de-fotos/)

Biological characteristics

- Oval body, rather deep and compressed.
- Regularly curved head profile.
- Small eye.
- Low mouth, very slightly oblique.
- Thick lips.
- 4 to 6 canine-like teeth in each jaw, followed by blunter teeth.
- 1 dorsal, 1 fork-like caudal and 1 anal fin. 2 pectoral and 2 pelvics fins.
- Scaly cheeks.
- Silvery grey color with a black blotch along the lateral line. A golden frontal band between eyes edged by two dark areas. Redish cheeks and dark areas at the edge of the caudal fin.

Biology and habitat

- Mediterranean Sea, Eastern Atlantic coasts and the Black Sea (Bauchot and Hureau, 1986).
- Euryhaline and eurythermal habits
- Present in both marine and brackish water environments
- Breeding period: October-December
- Breeding: open sea

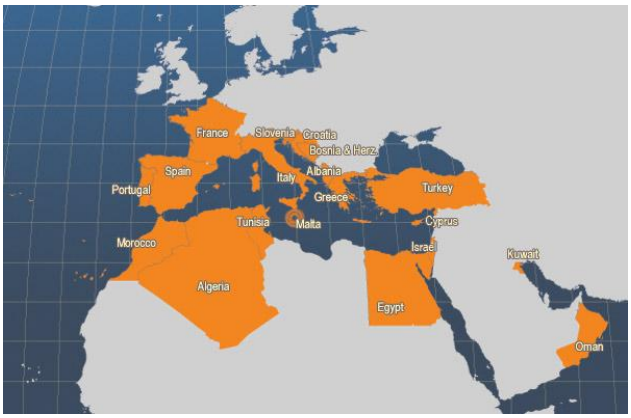

Figure 3: Map of the gilthead sea bream producer countries. (http://www.thefishsite.com/articles/1006/cultured-aquatic-species-gilthead-seabream)

- Juveniles: migrate towards protected coastal waters
- Adults habitat: rocky and seagrass (*Posidonia oceanica*) meadows, rarely sandy grounds
- Very sensitive to low temperatures (lower lethal limit is 4 °C)
- Reaching depth: <50 m
- Sexual characteristic: protandrous hermaphrodite
- Sexual maturity: in males at 2 years of age & in females at 2-3 years
- Breeding type: batch spawners (20 000-80 000 eggs every day for a period up to 4 months)

- Total length: <70cm
- Maximum published weight: 17.2 kg
- Maximum reported age: 11 years
- Feeding habits: Mainly carnivorous, accessorily herbivorous

History of production

First artificial breeding of sea bream was succeeded in Italy in 1981-82. At the late 1980s, Spain, Italy and Greece had also achieved production of gilthead sea bream juveniles. Intensive culture of the species was almost directly performed as there were no particular impediments. Indeed, more countries were continuously entering the sea bream industry every year. Production was increasing annually, providing (mainly in Mediterranean) aquaculture a profitable resource. As an example, sea bream production reached 87000 tons in 2000.

Commercial sea bream farming facilities

Extensive system

- Physical reproduction of the bloodstock
- Natural or remotely controlled rearing conditions (green water)
- Natural feeding in the rearing facilities
- Low fish densities
- Scarce human interference on the production
- Reaching commercial size (350g) in 20 months

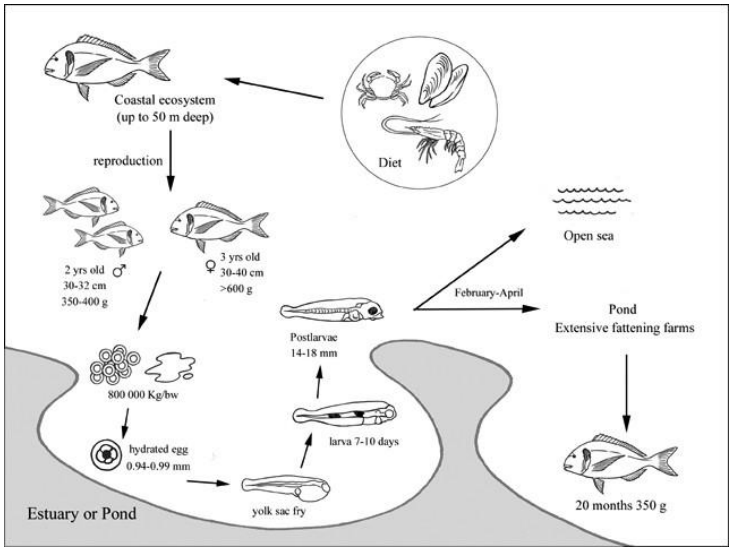

Figure 4: Production cycle of Sparus aurata - extensive system. (http://www.thefishsite.com/articles/1006/cultured-aquatic-species-gilthead-seabream)

(Dhert et al., 1998)

Intensive system

- Manipulation of breeding using hormones or artificial environmental conditions
- Absolute control of the larvae rearing conditions
- Exclusive exogenous feeding (Rotifer – Artemia – commercial feed)
- High fish densities
- Intense human interference on the production
- Reaching commercial size (350g) in 12 months

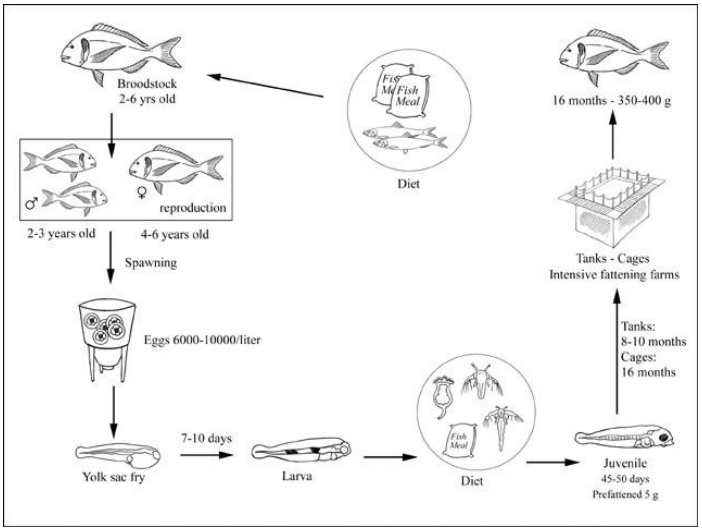

Figure 5: Production cycle of Sparus aurata - intensive system. (http://www.thefishsite.com/articles/1006/cultured-aquatic-species-gilthead-seabream)

(Dhert et al., 1998)

Rearing process of sea bream

Seed production and larval rearing still form a bottleneck for all on-growing operations. In addition, together are considered as the main limiting factors for industrial development (Dhert et al., 1998).

The whole procedure initiates with egg collection from broodstock tanks. Viable eggs are separated from sinking dead eggs and transferred to the incubator tank until hatching. For the first three-four (depending on the temperature) days post hatching (dph), sea bream larvae are fed by depleting the yolk-sac and the lipid droplet (**endogenous feeding**). Following, eyes are pigmented and the mouth developed (**mouth opening**), allowing the larvae to prey on live feed (**exogenous feeding**). The type of live feed is strongly related to the rearing technology used in aquaculture.

The technology of Mesocosm (extensive/semi-extensive rearing system) systems consists of a multispecies, natural food chain of microalgae (*Chlorella sp*. etc), zooplankton (tintinnid ciliates, *Synchaeta* and *Brachionus* rotifers, copepods etc.) and predators (fish larvae). The rearing conditions are both natural and artificial and thus, they are independent of any climatic and/or seasonal changes (Papandroulakis et al., 2004).

In intensive systems, rearing conditions are fully controlled and manipulated. Rotifers (*Brachionus plicatilis*) and *Artemia salina* nauplii are the main animals that are provided during larvae feeding. Both animals have been previously enriched with commercial lipid preparations, to enhance their levels of certain essential fatty acids and vitamins that are critically important for better growth, development and survival of the fish larvae. Rotifers are regularly used up to 10-12dph, according to the rearing temperature, while replaced by *Artemia* until the metamorphosis stage (32-35dph). Live feed integration very often coincides with the onset of notochord flexion.

Microalgae (e.g. *Chlorella sp*., *Isochrysis galbana*, *Pavlova lutheri*, *Nannochloropsis oculata*, *N. gaditana*, *Dunaliella tertiolecta*) are widely used in the rearing process, including the reproduction and maintenance of live feed as well the enhancement of water quality in the larval tanks. Nowadays, the supplementary microalgae in the water, also called “pseudo-green” water (Papandroulakis et al., 2001) is widely used in the initial rearing process, aiming to produce high value fish at elevated stocking density.

Fish larvae foraging behavior towards live feed

Quantitatively describe the foraging behavior of fish larvae towards live feed is of great importance in aquaculture, since it allows us to understand the efficiency and success of feeding process.

In natural habitats, fish foraging behavior is divided into four district actions (Barnabe, 1974, Gerritsen and Strickler, 1977, Kentouri, 1985):

- Encounter between prey and predator
- Potential prey recognition and attack of the predator
- Prey capture
- Prey ingestion

Regarding larvae rearing process, research should be focused on the potential effect of several factors (temperature, stocking density, food supply, light intensity, water turbidity, use of green water, etc) on larval ability to locate and capture its prey as well as how these dynamics change due to fish ontogeny.

Several studies have already been demonstrated the effect of temperature on fish larvae metabolism (Pechenik, 1987; Pechenik et al., 1990; Boidron-Metarion, 1995) (Houde, 1987, 1996). Consequently, the control of temperature during the rearing process could lead to the manipulation of the physiological responses that are responsible for increasing fish larvae development.

Stocking density is also associated with the larval foraging behavior, since lower density as well as sufficient amount of food, complies with better growth performance.

Studies have also shown that most marine fish larvae, including sea bream larvae, are visual feeders (Blaxter and Staines, 1970, Hunter, 1981 and Blaxter, 1986) and therefore unable to consume during the dark phase (Kentouri, 1985,Tandler and Helps, 1985 and Chatain and Ounais Guschemann, 1991). Light plays an important role in larval fish growth and survival (Blaxter, 1975, Batty, 1987, Puvanendran and Brown 2002). In addition, it can influence the foraging behavior of fish larvae that includes swimming duration and capture success (Puvanendran and Brown 2002). Similar effects have been observed in culture conditions, where light regimes induces a rapid start of feeding activity (Gulbrandsen 1991; Huse 1994). Particularly, the first-feeding phase requires a "threshold" light intensity to initiate feeding (Blaxter, 1986). As compared to 18-h photo phase, continuous illumination increases the maintenance cost of fish larvae and so, resulted to different growth performance (Papandroulakis et al., 2002).

It has already been documented that the feeding performance is related to the larval age and light intensity, regardless the rearing condition (clear-, green water). Nevertheless, the most notable effects of adding algae (pseudo-green water) to the rearing medium are the increased survival rate

(Papandroulakis et al., 2002) and the increased prey ingestion during exogenous feeding (Baskerville-Bridges et al., 2004; Shaw et al., 2006). Reitan et al. (1997) suggests that the supplementary use of microalgae when feeding with Rotifers resulted to a higher consumption of Rotifers and thus, to a higher growth and a better survival rate (Reitan et al., 1993, 1994; Oie et al., 1996) as compared to that of clear water (Reitan et al., 1993). Moreover, the combination of microalgae presence and modified light conditions results to an increased feeding rate on the live feed (Naas et al., 1992). However, the positive effects of algae on larval feeding are species-specific and also they depend on physical factors like algal concentration and light intensity (Baskerville-Bridges et al., 2004; Carton, 2005).

Aim of present study

The objective of the present study was the performance of two successive experiments under fully-controlled laboratory conditions to:

1. Study possible differences in foraging behavior in relation with 3 distinct rearing mediums.
2. Describe the prey-capture behavioral pattern.
3. Establish a rearing protocol of sea bream larvae in experimental tanks through the ontogenetic phases of mouth opening, onset of flexion, completion of flexion (2 experiments).

MATERIALS AND METHODS

The experiments were performed at the laboratory aquaculture facilities of the University of Crete. The first experiment initiated on the 9th of November 2012 and was completed on the 20th of December 2012, whereas the second one started on the 25th of February and last until the 12th of April 2013.

Experimental design for larvae rearing

Experiment 1

Nine polyester, light blue, rectangular tanks were used for the two experiments (115 cm x 35 cm x 40 cm) with 100 lt capacity each. The tanks

were oriented in a 3 x 3 formation. Each column of the array represented a triplicate, while differentiated in rearing medium:

- Tanks: 70lt artificial sea water.
- Water salinity: 38-40‰.
- Temperature: 20 ˚C.
- Photoperiod: constant by a fluorescent tube (30 Watt) above each tank.
- Aeration: air pump and 18 air stones, 2 in each tank.

Egg hatching

A total number of 50000 sea bream eggs were provided from the Hellenic Center of Marine Research (HCMR), in Gournes, Herakleion. A 50 lt plastic bag enriched with >100% saturated oxygen was used to transfer the eggs. The eggs were at the late gastrula stage in order to increase the survival rate during the transfer from HCMR to UoC Biology Department.

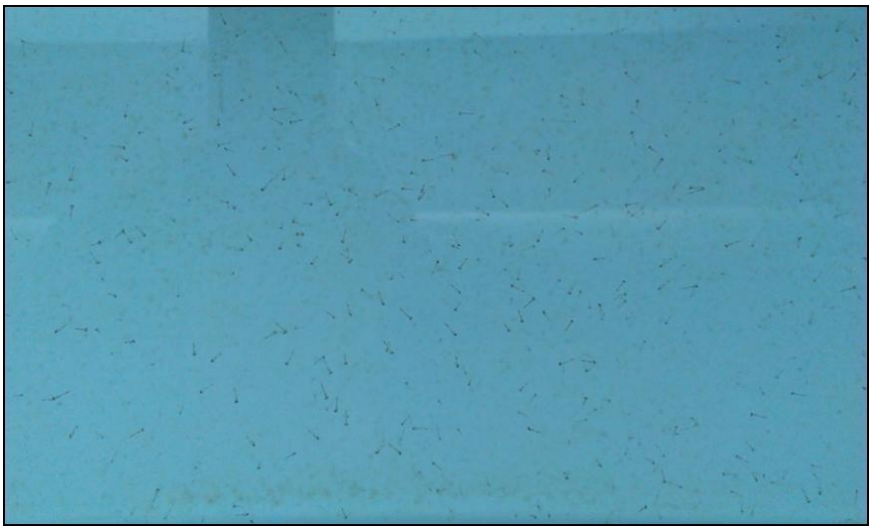

Figure 6: Fish were endogenous fed by their yolk sack and the lipid droplet until dph3. From dph4 (mouth opening), rotifer and Artemia were provided respectively. In addition, the tanks were differentiated with the rearing mediums

An acclimatization period took place before eggs were introduced to the rearing tanks. Eggs were equally distributed in 9 (9 x 5000eggs) plastic beakers (2 lt) for a period of two hours. A small amount of the tank water (100 ml) was being added in the beakers every 20 minutes to simulate the tank conditions (temperature & salinity). After acclimatization, the eggs were slowly and gently introduced into the tanks. Strong aeration was initially performed to

prevent eggs from sinking to the bottom, which would pose a big risk in terms of physical stress and bacterial infection. Hatching started 1 hour afterwards and lasted for 7 hours. After hatching, aeration was reduced to avoid stress condition to the newly hatched larvae.

Figure 7: Tanks were oriented in 3x3 formation. Each column referred as a triplicate, for statistical purposes, each representing one of the three rearing mediums.

Prior to any exogenous feeding, larvae nutritional requirements were covered by the absorption of the yolk sack and the lipid droplet. During this period, clear water was used for all of the 9 tanks (Figure 6). On dph 3, mouth opening was completed and the experimental procedure was differentiated.

Tank mediums preparation

The first column contained the Phytoplankton Medium (PM). The second column was in the middle, containing the Clear Medium (CM). CM was the control with clear water. The third column contained a solution of diatoms powder- Diatom Medium (DM) (Figures 7 & 8).

Microalgae, *Chlorella minutissima*, was provided from HCMR. The concentration given was ~200 x 10^6 cells per ml. In order to achieve the regular 30 NTU turbidity, 1.5 lt of microalgae solution was added in every tank of the PM, forming a green-color environment. In the tanks of the CM, no medium was added. The same water turbidity of 30 NTU in the DM was implicated by 2.1 gr of diatom powder, diluted in a beaker with 200 ml of tank water (30mg/lt), creating a milky-like color.

Figure 8: Sea bream larvae at dph 15 in (a) diatoms, (b) clear water, (c) phytoplankton . Photos are taken from the analysis custom made application. Larvae are clearly observed in high detail from the acquired frames of the application.

The maintenance of mediums' turbidity was accomplished by adding the relevant solution twice per week throughout the experiment. Water renewal (10%) was also performed once per week, while profile measurements (O_2 saturation, temperature and salinity) were controlled daily.

Experiment 2

The second experiment was conducted in the same experimental tanks and almost similar laboratory conditions. Particularly,

- DM rearing medium changed to a chlorophyll powder solution- Chlorophyll Condition (ChM).
- An additional (3rd feeding period) was performed.
- By the end of the experiment (5 last days) larvae were developed enough to accept commercial food.

In addition,

- Tank formation (3 x 3) differentiated so, every raw and column contained tanks of all the 3 mediums.
- Once per week NO_3^-, NO_2^- and NH_4^+/ NH_3 tests were performed in every tank to measure and control the water quality.
- Renewal of the water was accomplished once per week, reaching 20%.
- To eliminate probable stress to larvae from the light sharpness, scattering material was used, covering the lights above the tanks.

Feeding procedure

A plastic, 10lt (288 x 106 cells/ ml) bottle of phytoplankton (*Chlorella minutissima*) was preserved under strong aeration, constant illumination and temperature at 23-24˚C for the need of the experiment. Phytoplankton was

used for live feed enrichment and also the maintenance of the PM in the relevant tanks.

Exogenous feeding initiated at dph 3, with the completion of mouth opening. The regular concentration was 3-5 Rotifers / ml of tank water. Therefore, approximately 2.5 x 10^5 Rotifers per tank were given every feeding time. Before feeding, Rotifers (*Brachionus plicatilis*) were enriched with Ωmega-Enrichment (Foresee Management), to acquire the entire nutrient necessary for larvae growth and survival. Feeding was run twice every day, one at 09:00 - 10:00 in the morning and the second at 18:00 – 19:00. Therefore, daily needs were approximately 5 x 10^6 Rotifers. The amount of Rotifers (Figure 9) was estimated before the feeding process. For the needs of the experiment, Rotifer culture (10 x $10^{6)}$ was kept in 10lt plastic bottles with phytoplankton and strong aeration and plenty of illumination.

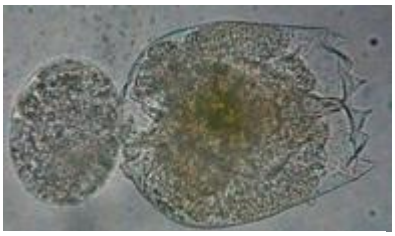

Figure 2: Rotifer, Brachionus plicatilis. (http://irrec.ifas.ufl.edu/aquaculture/research/research.html)

The feeding procedure was continued with *Artemia* nauplii (*Artemia sp*) at dph 20 (Figure 10). Approximately 8000 *Artemia* nauplii were given in every tank once every day. Unshelled *Artemia* cysts were provided from HCMR. Preserve of the cysts was accomplished at 4˚C. The day before feeding, 2 ml of *Artemia* cysts (10000 cysts/ ml) were introduced in the *Artemia* hatching device (24 hours). After hatching, *Artemia* nauplii were kept in a beaker (2lt) for 6 hours before enrichment and 12hrs before feeding.

Figure 10: Artemia nauplii (http://www.artemiaworld.com/home)

Larvae sampling

Ten larvae individuals per treatment were sampled every two days of the experiment to evaluate the growth performance in each rearing medium. Larvae were placed in different beackers (30ml), according to the rearing medium and then:

- They were anesthetized
- Total body length was measured using a stereoscope
- Photos of larvae were taken by a camera embedded to the stereoscope.

Larvae observation

A low cost, digital recording computer-vision system was developed to monitor sea bream larvae behavior. The features of the system are described below (Figure 11):

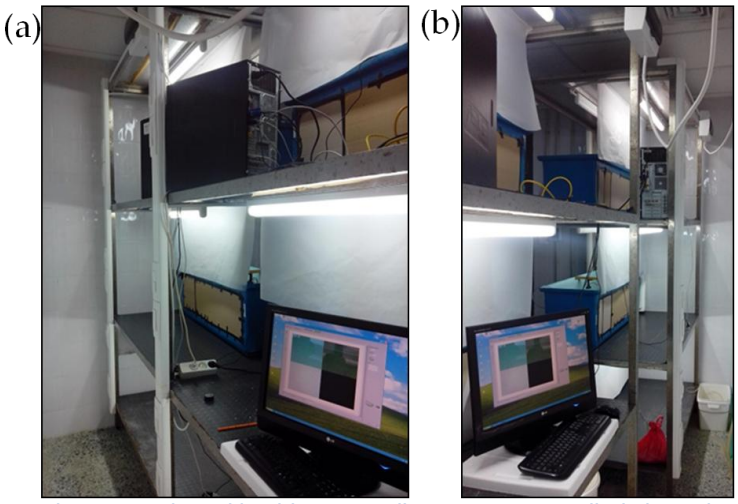

Figure 11: (a & b) Video recordings were controlled through a single monitor and keyboard/mouse, connecting to the three computers. Light diffusion was achieved by scattering material in front of the lights.

- Nine color digital CCD cameras (HD Webcam C310, Logitech)
- Data acquisition rate was set to 10 frames per second
- Imaging spatial resolution <100um per pixel
- Field of camera recording: 3.7 x 5.6 cm
- DivX 4.12 compression

Figure 3: Cameras were placed 8 cm upon the water surface.

Recordings occurred twice every day at the feeding time (9-10 και 6-7). Video data were stored automatically on the computer HDD. For the purposes of the experiment, cameras were placed upon each tank (~10cm) to get the maximum cover area and the best image quality of fish larvae. The recording ability of the cameras with absolute resolution reached 3cm below the water surface (Figure 12).

Data analysis

The video recordings were analyzed with the use of a custom made application developed in our lab. The analysis performed for 6 up to 40 days post hatching (dph), related to the developmental stages. Particularly, analysis refers to the following days:

- 5 and 6 dph (mouth opening)
- 20 and 21 dph (approximately urostyle bend up).
- 35 and 36 dph (flexion).

The foraging behavior of larvae was analyzed for a specific time period (½ hr), after the feeding time (9:30-10:00). In detail, the number of attacks was measured in each rearing medium, and then the type of attack was recognized. In addition, the number of larvae in the recording field area was measured on average, in 7 specific time periods (every 3000 frames). The analysis resulted into the number of attacks per individual for each tank.

An attack incident was referred to an action that larvae recognize – locate – attack – and capture its prey. The three different types of attack are described below:

- “S” type of attack
- “comma” type of attack
- And “burst” type of attack

The specific posture of the larvae body while locating and attacking to the live feed, and it was quite similar to the letter S, was referred as “S” type of attack. Further, as “comma” was referred the posture, that was quite similar to the punctuation mark “comma”. As “burst” were recorded the attacks with no special body posture but when larvae located the live feed and attacked straight forward.

RESULTS

Experiment 1

Growth performance

Larval development was documented for a period of 41 days in the 3 mediums. At dph 3, eyes pigmentation and mouth opening occurred and live feeding initiated with Rotifers. Throughout the days, we successfully recognized the appearance of chromatophores, gastrointestinal system, swim bladder and spleen of larvae. Later, feeding with *Artemia* was introduced at dph 20, with the initial phase of the flexion and the raise of urostyle (~25 dph). By the end of the experiment, there were numerously well formatted larvae having reached the completion of the flexion phase (figure 13).

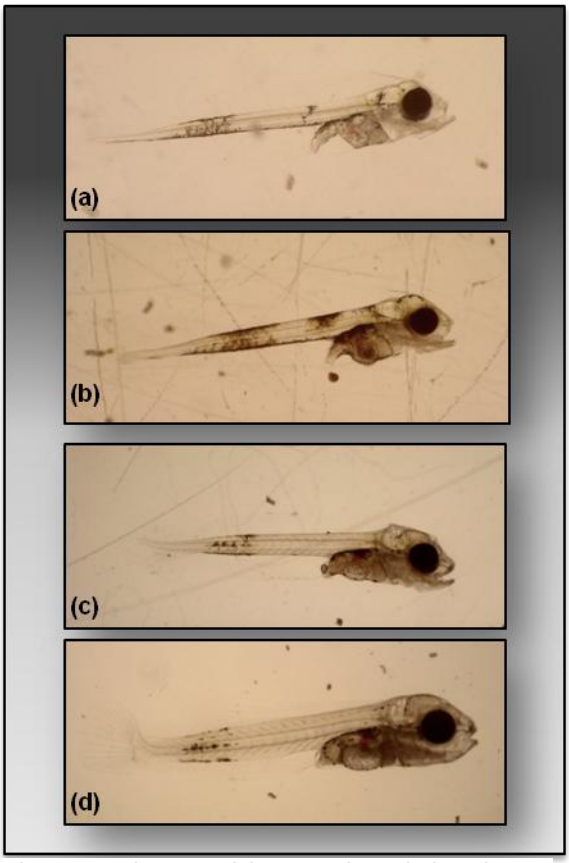

Figure 4: Pictures of larvae taken during the 1st experiment: (a) – 12th dph, (b) – 14th dph, (c) - 27th dph, (d) – 37th dph.

However, growth rate and ontogeny presented significant differences among the rearing mediums. Larvae reared in PM performed slightly better than larvae reared in DM. In contrast, in CM larval development was slower as compared to PM and DM, while mortality rate was higher.

A significant increase in larval growth rate was observed by the time that feeding changed to *Artemia* (figure 14).

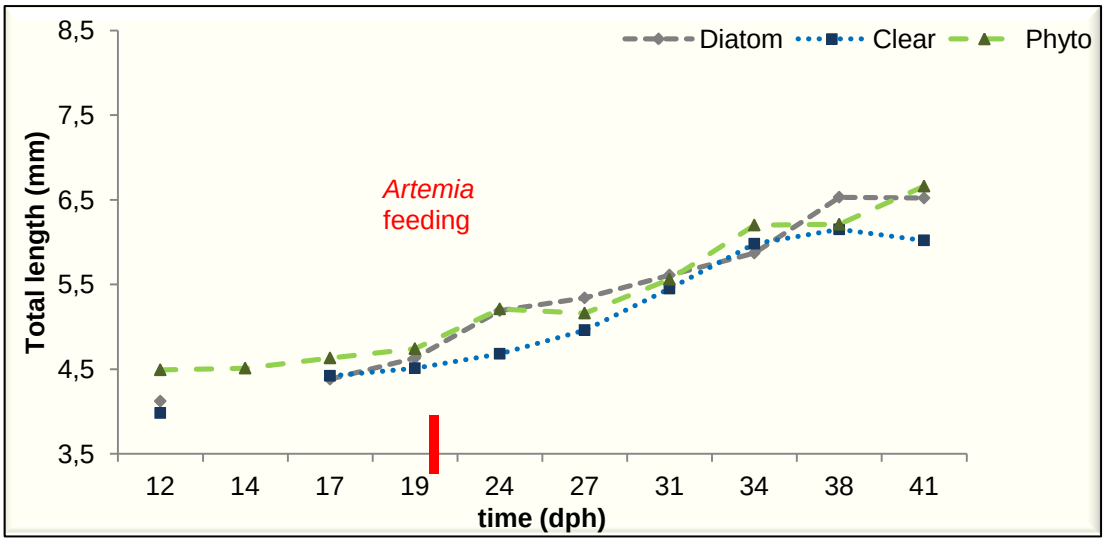

Figure 5: Sea bream larvae growth performance throughout rearing period. Data referred to the average of larvae TL among the 3 different mediums.

Foraging Behavior estimation

In general, sea bream larvae foraging behavior was more intense in the PM than the other two mediums. Detailed analysis through the days showed that PM and DM presented equally high values early in the experiment as compared to the CM. Following, the number of attacks that referred to PM was twice as the relevant number of attack incidents of CM and DM, respectively. By the end of experiment, almost no attacks were recorded at the CM. In addition, number of attacks on PM was still higher than the relevant number on DM (Figure 15). It should be mentioned that the number of attacks per individual referred to the ½ hour time period of the analysis.

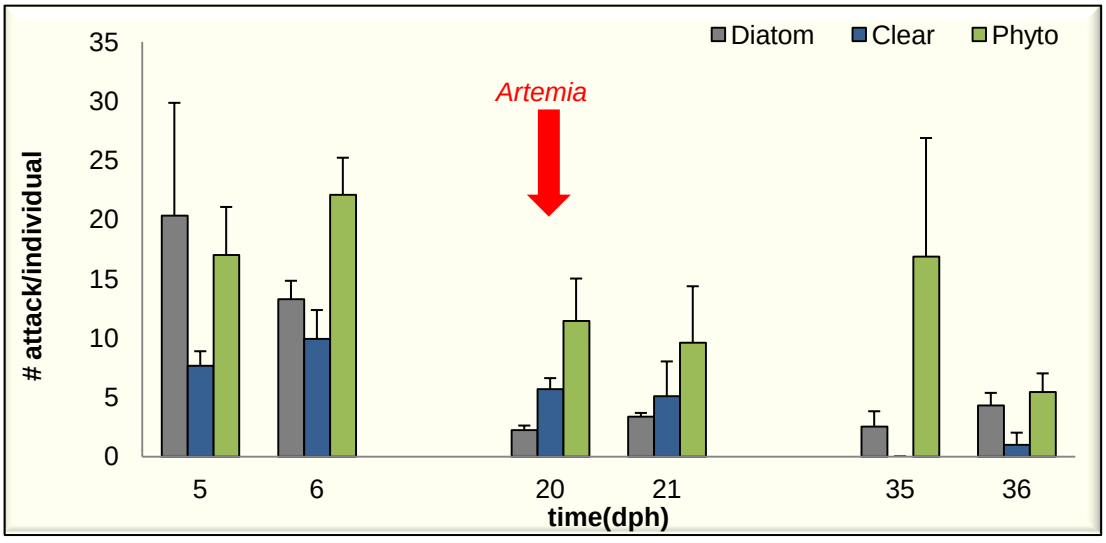

Figure 6: Number of attacks per individual regarding the six days of analysis. Data referred to the average (± SE) number of attack incidents measured per rearing medium.

The following figures (16, 17 & 18) present the evolution of foraging behavior for the three rearing mediums, respectively.

The number of attacks at the DM was significantly higher at the beginning of the experiment whereas, they present a remarkable reduce in the middle (dph 20 & 21) and at the end of the experiment (Figure 7).

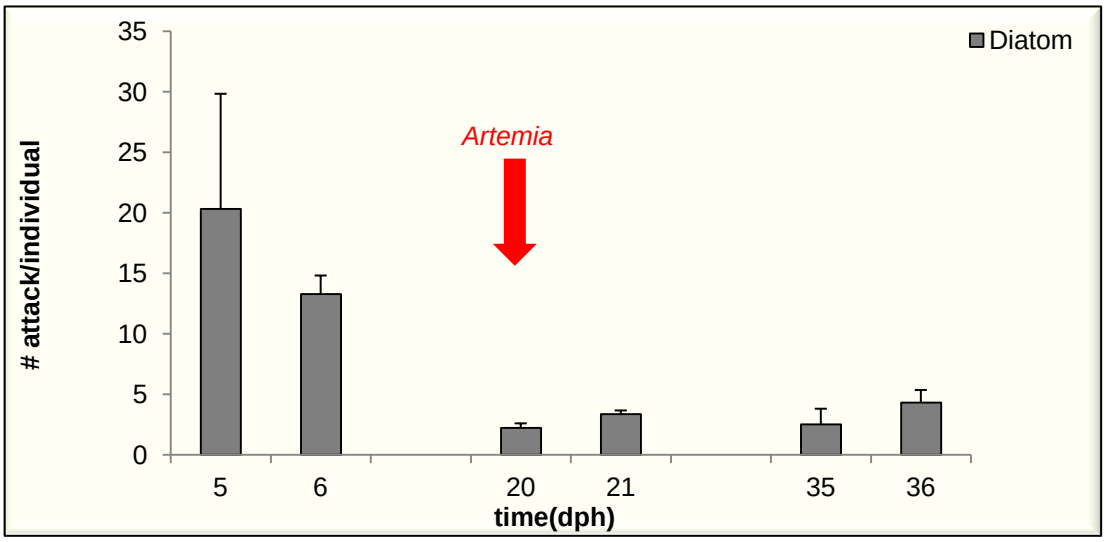

Figure 16: Number of attacks per individual regarding the six days of analysis. Data referred to the average (± SE) number of attack incidents measured in DM.

The number of attacks at the DM at the beginning and in the middle of the experiment presented no significant difference. Moreover, at the later experimental days almost no foraging behavior performance was recorded (Figure 17).

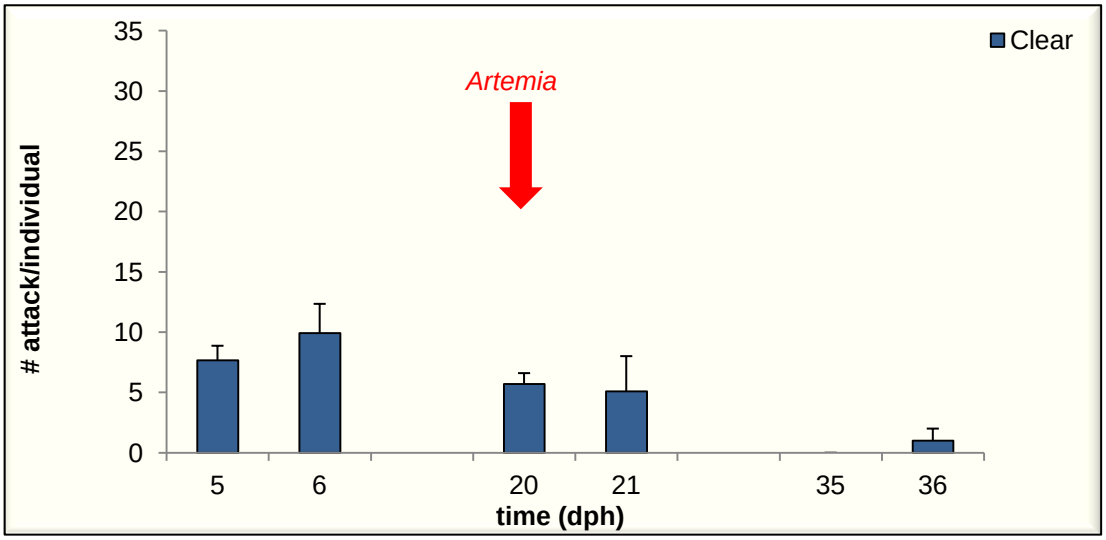

Figure 8: Number of attacks per individual regarding the six days of analysis. Data referred to the average (± SE) number of attack incidents measured in CM.

The number of the attack incidents at the PM was remarkably high at the beginning of the experiment while at the middle of the experiment and at the end it showed a significant drop compared to the first experimental days (Figure 9).

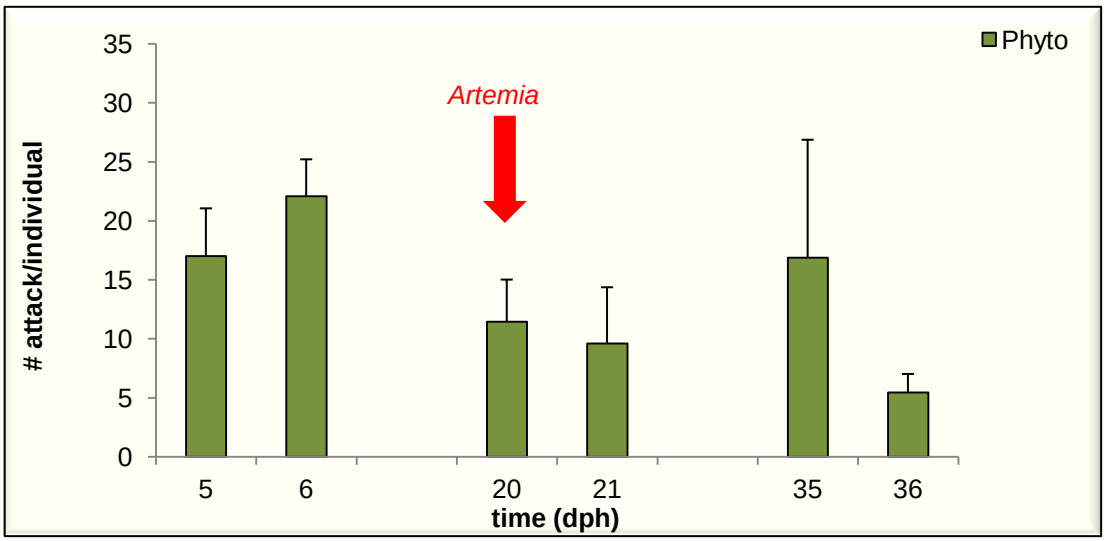

Figure 18: Number of attacks per individual regarding the six days of analysis. Data referred to the average (± SE) number of attack incidents measured in PM.

Figure 19 demonstrates that the type of attack is strongly related to the larval growth. The initial “S” and “comma” type of attack have been replaced by “burst” type during the experiment.

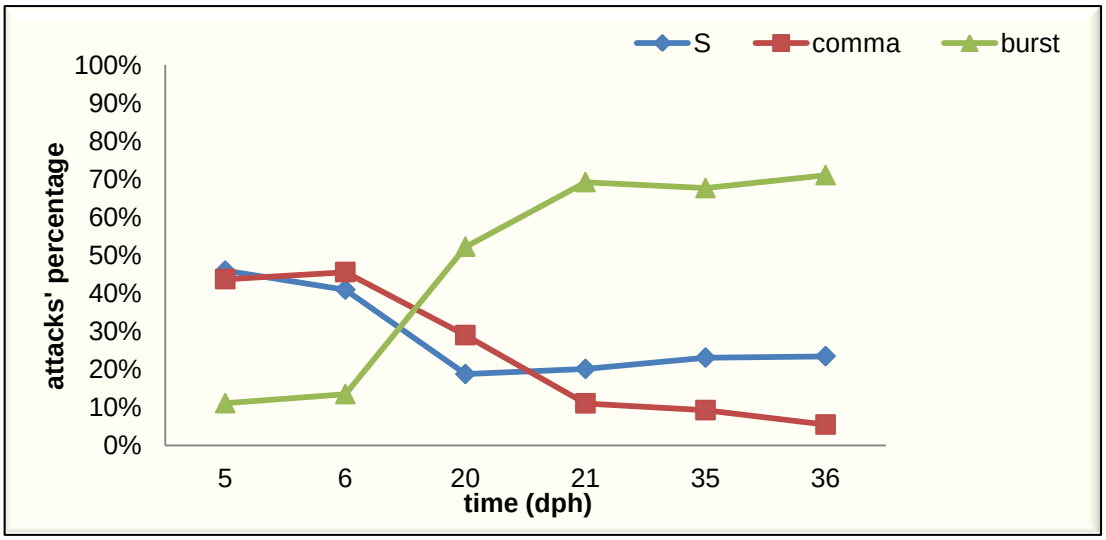

Figure 10: Percentage of the type of attack regarding the six days of analysis. Data referred to the percentage of each attack type for all the rearing mediums.

In the figures below (20, 21, 22), a detailed analysis of the attack pattern is presented for each medium, throughout the experiment. In PM, sea bream larvae behavior changed from “S” and “comma” to “burst” type of attack faster and more clear than the remaining two mediums (DM, CM).

Initially (5 & 6 dph), the majority of the attack incidents at the DM were “S” and / or “comma” type of attack. Further, on 20 and 21 dph, “burst” type of attack increased significantly whereas, “S” type but especially “comma” type declined. At the end of the experiment “burst” type of attack percentage remained at a higher level compared to the other two types, while “S” attack type percentage increased slightly and the “comma” type percentage remained at a low level.

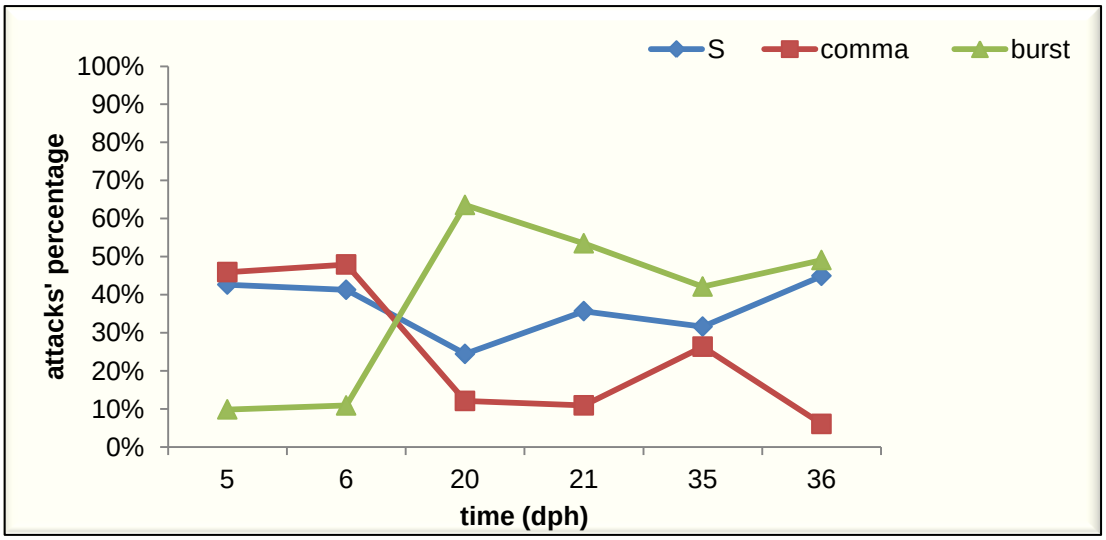

Figure 20: Percentage of the attack type out of the total attack incidents regarding the six days of analysis. Data referred to the percentage of each attack type for DM.

The attack's percentage of "S" and "comma" type of attack was significantly higher at the beginning of the experiment at the CM compared to "burst" type of attack. From the middle of the experiment the "burst" type of attack percentage increased gradually until the end of the experiment (no attack incident recorded on dph 35) while both "S" and "comma" type of attack percentage declined significantly from dph 20 until the later experimental days (Figure 11).

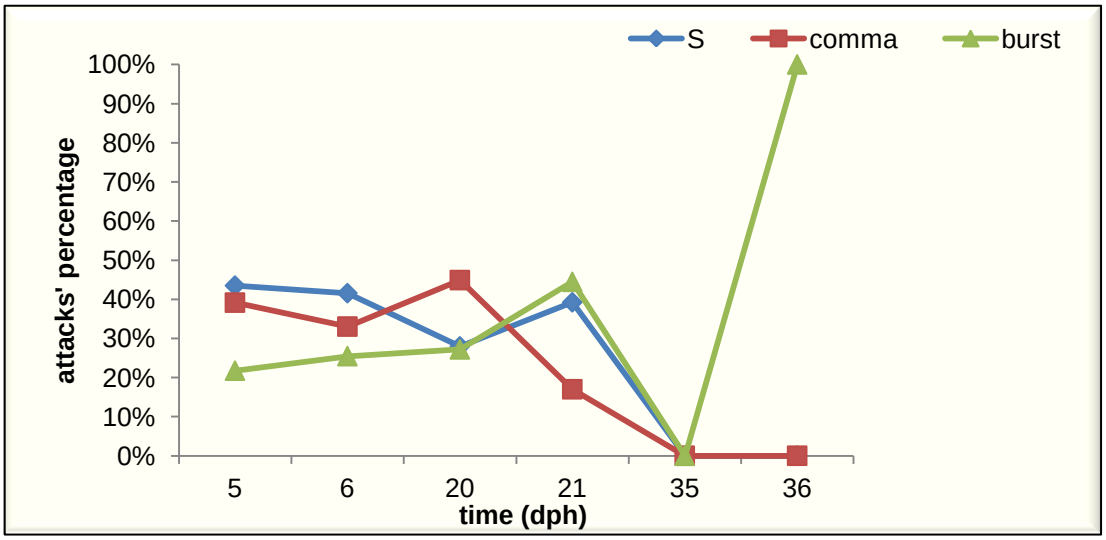

Figure 21: Percentage of the attack type out of the total attack incidents regarding the six days of analysis. Data referred to the percentage of each attack type for CM.

The low levels of the "burst" type of attack at the early experimental days of PM give way to a remarkably higher percentage at the middle and at the later days of the experiment. In contrast, equally high percentage of "S" and

“comma” type of attacks at the beginning of the experiment present a significant continuous decrease until the end of the experiment.

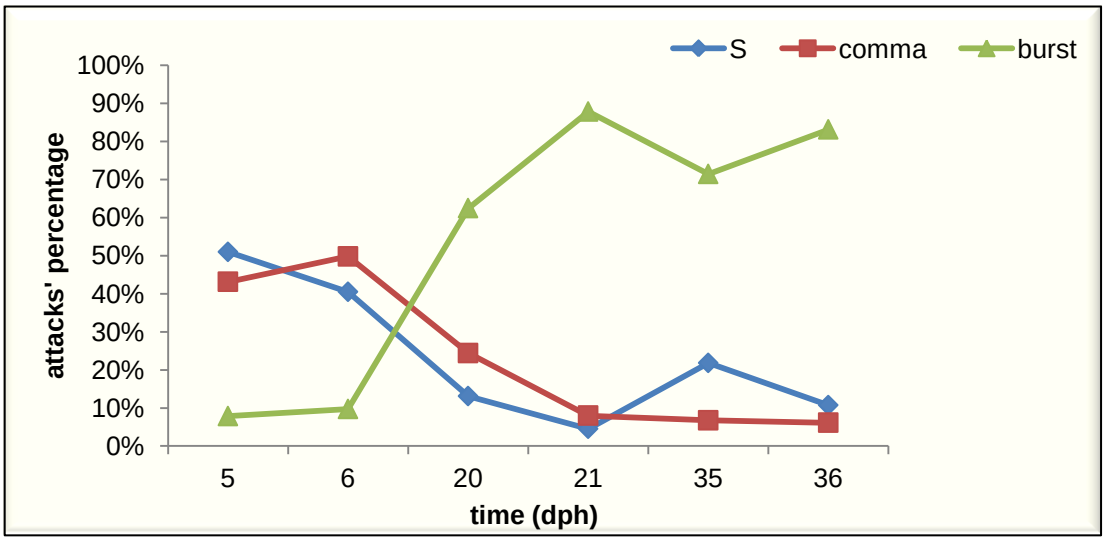

Figure 22: Percentage of the attack type out of the total attack incidents regarding the six days of analysis. Data referred to the percentage of each attack type for PM.

Cannibalism incidents were observed during the analysis of the acquired video data. Particularly, sea bream larvae were attacking each other, but no mortality was caused by this behavior.

Experiment 2

Growth performance

The enhanced protocol that was used in the 2nd experiment resulted to far better growth performance and ontogenetic development of fish larvae (Figure 23).

- Yolk sac and lipid droplet absorption
- Eye pigmentation
- Mouth opening
- Organic systems development (respiratory, gastrointestinal, nervous, muscular e. a.)
- Chromatophores development
- Vertebrate column development
- Caudal fin formation (flexion)
- Pectoral, dorsal, anal and pelvic fin formation

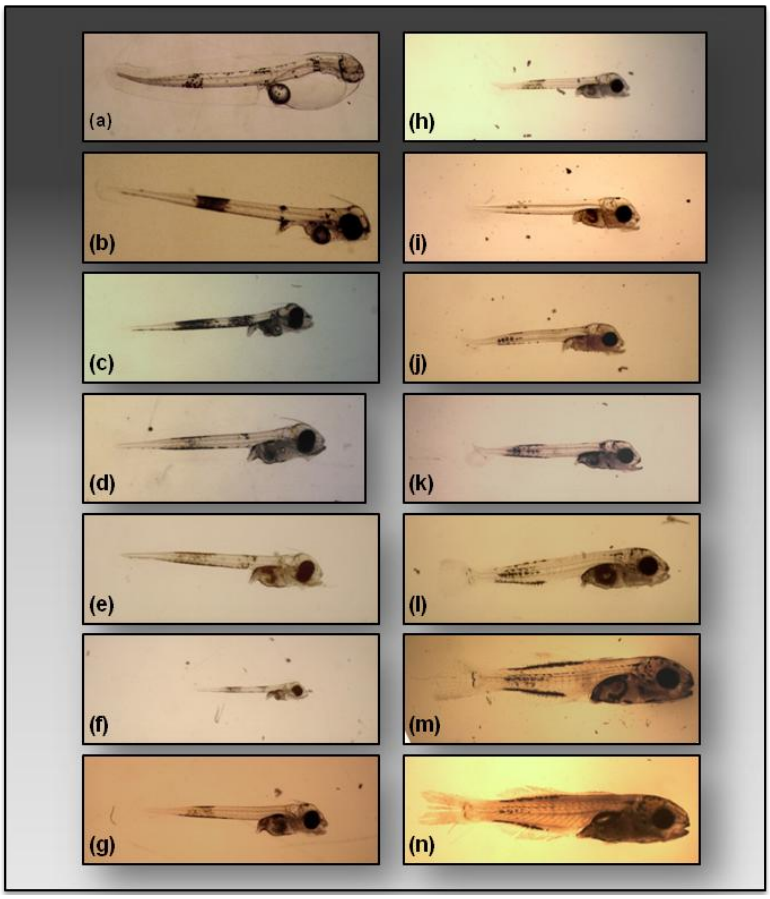

Figure 23: Pictures of larvae taken during the 2nd experiment: (a) – 3nd dph, (b) – 4th dph, (c) - 7th dph, (d) – 9th dph, (e) – 11th dph, (f) – 14th dph, (g) - 16th dph, (h) – 18th dph, (i) – 22nd dph, (j) – 25th dph, (k) – 29th dph, (l) – 35th dph, (m) – 42nd dph, (n) – 46th dph.

In the 2nd experiment, ChM performed equally well with the PM, presenting high larval growth rate. In addition, larval growth at CM was remarkably lower (Figure 24).

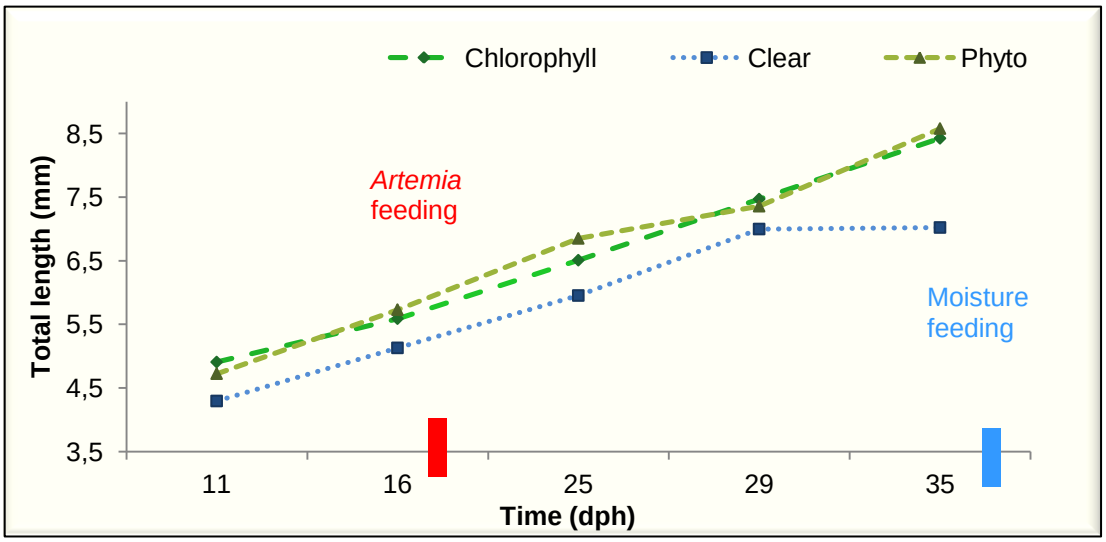

Figure 24: Sea bream larvae growth performance throughout rearing period. Data referred to the average of larvae TL among the 3 different mediums.

Comparison of the experiments

The better growth rate of fish larvae at PM and CM in the 2nd experiment is a combined result of both enhanced rearing conditions and three feedings (Figures 25 & 26).

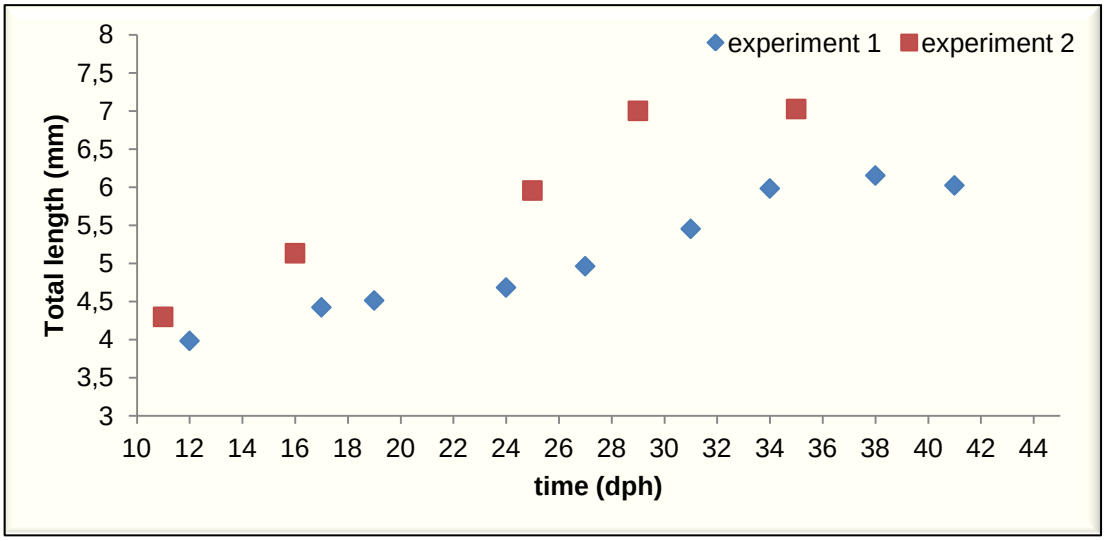

Figure 25: Scatter diagram of the sea bream larvae growth performance between the 2 experiments. Data refer to the larval daily average TL of the CM rearing medium.

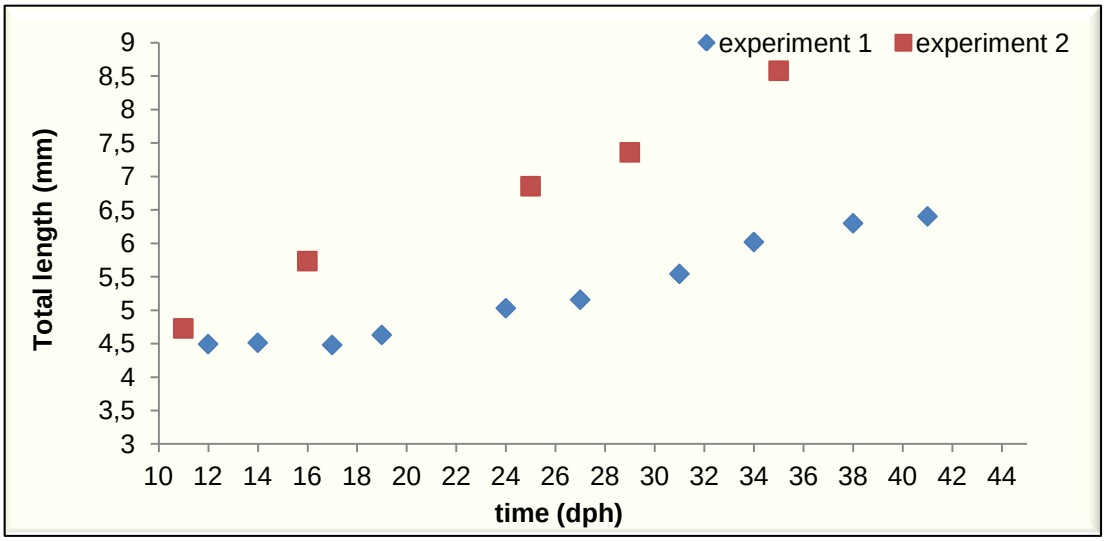

Figure 26: Scatter diagram of the sea bream larvae growth performance between the 2 experiments. Data refer to the larval daily average TL of the PM rearing medium.

DISCUSSION

The pattern of sea bream foraging behavior (locate, attack and capture of live feed) is firstly described in the present study. The decreasing number of attacks that was observed throughout the experimental days can be explained

by the results of both increased nutritional value and the fact that sea bream larvae were robust enough to consume the live feed through suction feeding. Especially on dph 20 and 21, the number of larvae on PM and DM recording field was really high (>15 larvae on the field). Therefore, a high attack rate in both mediums was expected. Contrary to our expectations, attack rate reduced in both mediums with a particular rapid reduction on DM, while CM was slightly affected. The related figure of growth performance indicates that larval growth on PM and DM could be higher than CM, thus, larvae from these mediums were able to capture *Artemia* (*Artemia* has higher nutritional value than Rotifer, as a consequence the energy gained with less effort) or/and they were able to capture rotifers not only by ram feeding but also with suction feeding. Related studies (Norton 1991, Norton and Brainerd 1993) in fish larvae have already described two feeding patterns, suction feeding and ram feeding, based on the morphological characteristic of the animal as well as the type of prey. This is the first study stating that sea bream also exhibits suction feeding even at 20 dph.

Results obtained clearly demonstrate that the rearing medium has a strong influence on the early development and foraging behavior of sea bream larvae. Furthermore, the additional feeding period (third) resulted to a higher growth rate in the 2nd experiment. The use of microalgae at the early developmental stages of fish larvae is a common procedure in aquaculture nowadays (Muller-Feuga et al. 2003b). When phytoplankton is included in larval rearing tanks, the survival, growth and food conversion index are better than in clear-water conditions (Howell 1979, Scott & Baynes 1979, Scott & Middleton 1979, Eda et al. 1990, Reitan et al 1993, Dhert et al 1998, Tamaru et al 1994, Papandroulakis et al. 2000, 2002a, b; Divanach & Kentouri 2000).

The significant differences between PM and CM that were observed during the two experiments come in agreement with related studies, where it is clearly demonstrated that microalgae background has an important, but specifically different, effect on the timing and intensity of first zooplanktonic feeding. In rearing trials, first (zooplankton) feeding time in Sparidae was earlier in green than clear water, and better matched the moment corresponding to mouth opening (Kentouri 1985). First feeding larvae of turbot

(Reitan et al. 1993, Øie et al 1997) and halibut (Reitan et al. 1997, Naas et al. 1992) showed an increased rate of rotifer's consumption *B. plicatilis* in tanks containing microalgae rather than clear water.

Phytoplankton particles change the turbidity and the light conditions in the water. Therefore, it influences the foraging behavior and the larvae prey ingestion (Naas et al., 1992 and Naas et al., 1996). The results of the present study are consistent with previous studies (Naas et al., 1992, Reitan et al., 1993, Tamaru et al., 1994, Cahu et al., 1998, Bengtson et al., 1999, Papandroulakis et al., 2002, Baskerville-Bridges et al., 2004 and Shaw et al., 2006), illustrating that larvae reared in PM had slightly better performance from DC and a far better performance than CM. Nevertheless, in the experiment 2 an equally well performance between PM and ChM was observed.

However, the use of microalgae in larvae rearing phase is species dependent. For those that are considered difficult to rear in clear water, such as halibut, turbot or sea bream, the gain is generally greater than 100 – 500 % (Naas et al. 1992, Reitan et al 1993, Papandroulakis et al. 2002a) and may exceed one order of magnitude (Scott & Baynes 1979). For species considered easy to rear in clear water, such as sea bass or mullet, microalgae enhances survival by 18 – 113 % (Tamaru et al. 1994, Cahu et al. 1998), which is sufficient to improve the cost-effectiveness of production.

Turbidity has been shown to enhance visually mediated feeding in some species (Benfield & Minello 1996; Rieger & Summerfelt 1997; Utne 1997; Cobcroft, Pankhurst, Hart & Battaglene 2001), while in others feeding performance has remained unaffected (Gardner 1981) or declined (Moore & Moore 1976). Feeding performance (incidence and intensity) of yellowtail kingfish larvae is influenced by light intensity and algal-induced turbidity. In our study, the foraging behavior of sea bream larvae in the DM was lower than in PM, since less attack events were recorded. Nevertheless, it can be assumed that the turbidity due to the diatoms solution had an effect on better visual recognition of live prey from sea bream larvae compared to the CM. As a consequence, sea bream larvae in DM presented a better growth performance.

In the second experiment, the use of chlorophyll solution in the water resulted to a similar growth rate performance as compared to the PM. Sea bream larvae had almost the same body length as PM. It can be assumed that the notable effect of microalgae presence on the water, regarding the light intensity and the water turbidity was also appeared in the ChM. Potentially, the strong factor of ChM, related to the high growth performance might be the green-color-environment created by the chlorophyll solution.

Microalgae also play a role in intestinal transit and gut repletion. Øie et al., 1997, found a greater number of rotifers in guts of turbot larvae in clear water than green water and suggested that a longer digestion time was responsible for better assimilation rate in clear water. Kentouri (1985) observed similar results for 2-5 DPF sea bream, noting that the 'gut was distended, giving the false impression of good feeding'. Part of distension was due to accumulation of empty rotifer lorica at the end of the rectum and a very low rate of excretion.

The addition of microalgae to rearing water modified the bacteriology of larval skin and gut (Skjermo and Vadstein 1993). Unlike skin microflora, which is clearly related to the flora of the water and less affected by algal addition than gut flora, the intestinal microflora of larvae kept in green water differed considerably from that of larvae kept in clear water. It consisted mainly of slow-growing bacteria, together with a smaller fraction of opportunistic bacteria (potential pathogens). Selection of bacteria in the gut was more active in green than in clear water, indicating that microalgae produce substances (e.g. lectins, taxins) that enhanced the ability of certain bacteria to grow in the gut.

The potential nutritional contribution of chlorophyll particles on sea bream larvae growth has to be further examined. Results could potentially introduce the use of chlorophyll solution in larviculture, since it is a cheaper and cost-effective product in comparison with the entire procedure of microalgae production.

CONCLUSION

- PM had better growth rate and foraging behavior performance
- CM is considered to be an inappropriate rearing medium for sea bream larvae
- DM turbidity has significantly lower effect on the foraging behavior than the relevant (PM) green color from microalgae.
- ChM, in the 2nd experiment had equal efficiency as the PM.
- The amount of rotifer provided to sea bream larvae should be taken into consideration since not enriched rotifers in tanks might have a negative effect on the structure of swim bladder.
- Cannibalism incidents were observed during the analysis of the acquired video data. Particularly, sea bream larvae were attacking each other, but no mortality was caused by this behavior.
- The type of attack is altered throughout the developmental stages of sea bream

Future developments

Strategic use of microalgae in hatcheries during the very early life of marine fish improves the success of first feeding, a prerequisite for efficient survival, growth and quality in fish larviculture. This phenomenon has led to a reconsideration of 3 mechanisms involved in early larval feeding:

- the role of drinking and filter-feeding during the transition from endotrophy to exotrophy
- the potential effect of dissolved organics from microalgae as contributors to feeding autonomy and anti-stress responses
- the pretrophic importance of microalgae as a trigger for both physiological and behavioral processes and bacterial probiotic conditioning of water, rotifers and larval gut.

In the future, improving the rearing conditions for the fish larvae will provide both a more hygienic environment and welfare combined with better growth and survival rates of reared larvae. Consequently, succeeding an improved juvenile and adult production, in terms of the quantity and the quality of the

product, leads to a profitable financial regime that affects not only the producer but also has a socio-economic impact.

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