Deliverable Report

Deliverable No:	D18.2		**Delivery Month:**	60
Deliverable Title	Determine optimum temperature conditions for rearing wreckfish larvae			
WP No:	18		**WP Lead beneficiary:**	P8. IEO
WP Title:	Larval husbandry - wreckfish			
Task No:	18.2. Subtask 18.2.1		**Task Lead beneficiary:**	P32 (MC2) and P8.(IEO)
Task Title:	Determine optimum temperature conditions for rearing wreckfish larvae			
Other beneficiaries:	P32. MC2	P19. CMRM	P1. HCMR	
Status:	Delivered		**Expected month:**	57
……..				

Lead Scientist preparing the Deliverable: Blanca Álvarez-Blázquez (IEO)

Other Scientists participating: Rodríguez Villanueva, J.L. (CMRM), Linares, F. (CMRM), Vilar, A. (MC2), Pérez Rial (IEO), E., Lluch, N. (IEO), Gómez Ceruelo, C., Martínez, J.M:, Montserrat Pérez (IEO).

Objective:

Water sea temperature is one of the most important exogenous factors for the eggs and larvae development (Kamler, 2002). Find an optimal temperature range for incubation and larval culture of wreckfish until approximately 60 dph (days post hatching) was the main objective. This protocol will be defined for each phase (embryonic development and larval culture) and the optimization will be evaluated from results obtained in larval survival, growth, deformities, pigmentation and vertical migration.

Background:

Wreckfish culture is an attractive option in order to further diversify commercial aquaculture in Europe. Its fast growth (Kentouri et al., 1995; Papandroulakis et al, 1997, Rodríguez Villanueva et al., 2014), easy handling in captivity, high market price, as well as the genetic homogeneity in the current stocks (Sedberry et al., 1999; Ball et al., 2000), making wreckfish an excellent candidate for the diversification of the marine aquaculture species in Europe.

The first reference on wreckfish natural spawns in captivity was published by Papandroulakis et al. (2004), who maintained a wreckfish stock during 8 years in which advances in sperm and oocytes quality were achieved. Large numbers of fertilized eggs were obtained in captivity by stripping followed by artificial fertilization leading to their culture under experimental conditions. This resulted in a detailed description of embryonic and larvae development until mouth opening (Papandroulakis et al, 2004).

Recently, Peleteiro et al (2011), obtained viable spawns by artificial fertilization with sperm and oocytes from Aquarium Finisterrae (MC2). Furthermore, a private company, Isidro 1952 (before 'Isidro de la Cal'), with facilities for wreckfish broodstock in Valdoviño (A Coruña), closed the cycle in 2013 with one individual that is actually in MC2, and recently (2018) 25 juveniles were obtained at Instituto Galego de Formación en Acuicultura (IGAFA,CMRM) facilities from Instituto Español de Oceanografía (IEO) and MC2 spawns.

Description:

FIRST TRIAL OF INCUBATION WITH TWO WATER TEMPERATURES

During May of 2016 trials with different incubation temperatures (14±0.5ºC and 17±0.5ºC) with eggs from broodstock of IEO facilities were carried out (**Fig.1).** The optimal incubation temperature was shown at 16±0.8°C. At this temperature the embryogenesis occurred in 4 days post fertilization (dpf) and hatching rate was 11.9%.

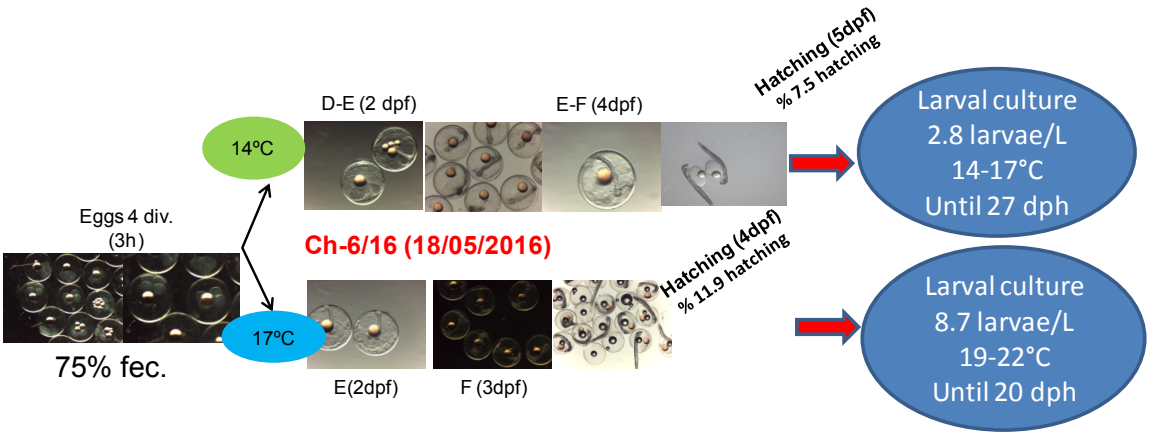


Figure 1. First incubation and larval culture trials with two spontaneous spawns of IEO (dpf = days post fecundation).

FIRST TRIAL OF LARVAL CULTURE WITH TWO WATER TEMPERATURES

Larval rearing trials in a 'green water system' were carried out with this spawn, using two temperature ranges: 16-18° C and 19-22° C in duplicate 500 L tanks. (**Fig. 9**)

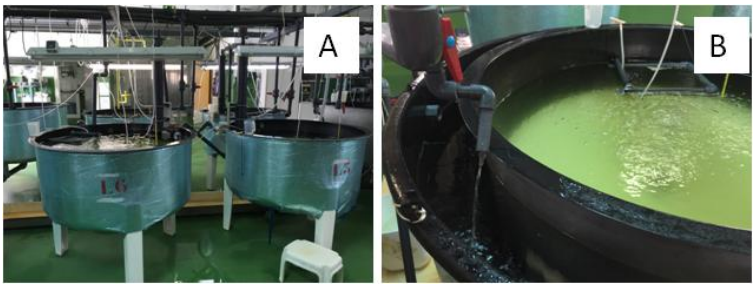


Figure 9. Larval culture tanks at 19-22ºC (A) and detail of the cooling system at 14-17ºC (B)

The initial densities were 8.7 larvae.l^{-1} at 20.7 ± 0.7°C and 2.8 larvae.l^{-1} at 16.2 ± 1.1°C. The tank volume was 450 L. Larvae reared at 20.7ºC did not demonstrate any food ingestion resulting in only 4 larvae alive at 20 dph. These were collected for biochemical analysis (CMRM). The larvae reared at 16.2ºC ate rotifers and Artemia (Álvarez-Blázquez et al. 2017), but did not exceed 27 dph. The last 3 remaining larvae were collected for biochemical analysis (MCMR). In both cases, natural photoperiod was maintained until the end of endogenous feeding. After that, 12 h of light (410 lux at the surface) was used. Samples of size and dry weight were taken in all the treatments every seven days from 3 dph to evaluate the growth.

In summary, there was no evidence that the food digestion occurred in these trials (**Fig 10**) as growth was negative (**Fig. 11**). Both length and dry weight decreased indicating that the surviving larvae were not properly fed while some of them also presented deformities. Nevertheless, the food acceptance was considered a success for these preliminary trials (Álvarez-Blázquez, 2016). On the other hand, ontogenesis of the digestive system is a slower process in wreckfish compared with other species. In fact, the ontogenesis of the organs related to the digestive and the vision system are not completed until 23 dph (see D1.1).

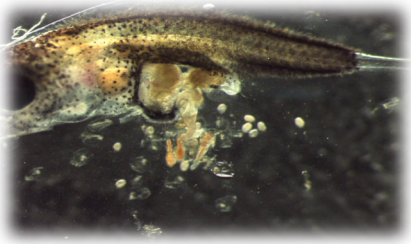


Figure 10. Larvae with 18 dph.. Digestive with artemia and eggs and rests of rotifer.

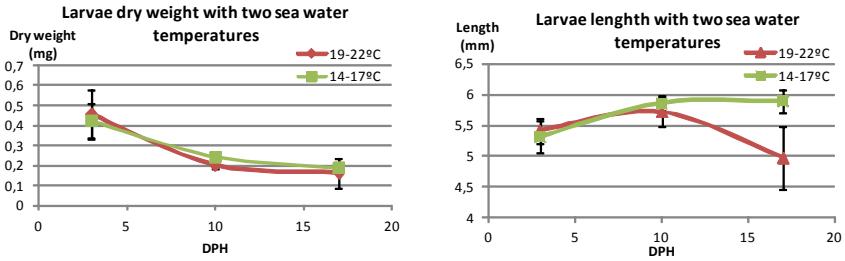


Figure 11. Larvae dry weight (mg) and length (mm) cultured in two different water temperatures (19-22ºC and 14-17ºC).

Despite the disappointing larval development results, these preliminary studies were very interesting for setting an optimal range of incubation temperatures in future experiments, but not sufficiently conclusive as the assays were not completed. Regarding larval rearing, the following data were verified:

- Larvae total length was 4.70±0.27 mm at 1 dph.
- Yolk sac was consumed by 11 dph at 14-17°C and by 8 dph at 17-20°C sea water temperature.
- Mouth opening occurred at 7 and 4 dph at 14-17°C and 17-20°C, respectively. Following mouth opening, larvae were fed with enriched rotifers and Artemia nauplii until 27 dph.

SECOND TRIAL OF INCUBATION WITH THREE WATER TEMPERATURES

During April 2017 an incubation experiment was carried out with three temperatures to extend and validate the results obtained during 2016. The temperature ranges were 13-14ºC, 16-17ºC and 19-20ºC. Floating eggs samples were taken from three spawns (one of which was finally rejected due to poor quality) between April 3rd and 17th, 2017. Approximately 1000 eggs (400 eggs.l^{-1}) were placed in triplicate 2.5 L cylindrical vessels with a 500µm mesh base **(Fig. 12)**. The water was filtered at 1µm and flow rate was 5-7 l.h^{-1} with soft aeration to facilitate egg mixing. The water temperature was recorded every three hours between 9:00 am and 9:00 pm everyday to verify that it was within the desired limits.

Figure 12. Methacrylate cylindrical vessels and tanks for egg incubation at three different water temperatures.

Each morning, a sample of 10 floating eggs was taken from each vessel to identify the stage of embryonic development and all the dead eggs from the bottom were removed and counted. All hatched larvae were counted, differentiating between those that were of good quality and those that had some type of deformity. With this data, the hatching and deformity rate for every temperature was calculated as follows:

$$\% \text{ Hatching} = N_L/N_E \times 100$$
$$\% \text{ Deformities} = N_D/N_L \times 100$$

Where N_L is the total number of hatched larvae, N_E the number of initial eggs and N_D the number of deformed larvae.

The average temperatures obtained during the experiment were 13.7 ± 0.2ºC, 16.6 ± 0.4ºC and 19.5 ± 0.4ºC. Embryonic development was different for each temperature, and lasted 4, 5 and 7 days at 19.5ºC, 16.6ºC and at 13.7ºC, respectively (Álvarez-Blázquez et al. 2017). The quality and number of individuals hatched is shown in the **Fig. 13**.

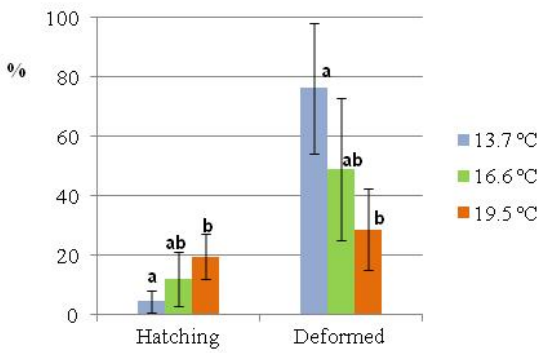


Figure 13. Hatching rate (average % ± SEM) and larvae quality (% deformed ± SEM) during the trials with three water temperatures.

Hatching increased with experimental temperature. At 13.7°C the hatching rate was 4.61% while at 19.5°C it improved to 19.7%. Conversely, larval deformity markedly dropped from 76.25% to 28.8% as the temperature increased.
Significant differences were observed ($p < 0.05$) in both hatching rate and deformed larvae between 13.7ºC and 19.5ºC, while there were no significant differences with respect to 16.6ºC. Therefore, the trend suggests that low temperature incubations promote very low hatching (%) and high deformity.
Regression analysis was performed (statistical program SPSS) to evaluate the trend of the data with respect to temperature (T) (**Fig. 14**). Several models were tested to obtain the regression curves that best fit our data (R^2 higher). The statistical program chose a potential regression model as the best solution to explain the variation in the percentage of hatching as a function of temperature, defined by the following equation:

$$Hatching\ (\%) = 1.17x10^{-7}x\ T^{6.401} \quad (R^2 = 0.527)$$

However, for the percentage of deformed larvae as a function of temperature a negative logistic regression was selected:

$$Deformed\ (\%) = -145.17x\ \ln(T) + 459.68 \quad (R^2 = 0.577)$$

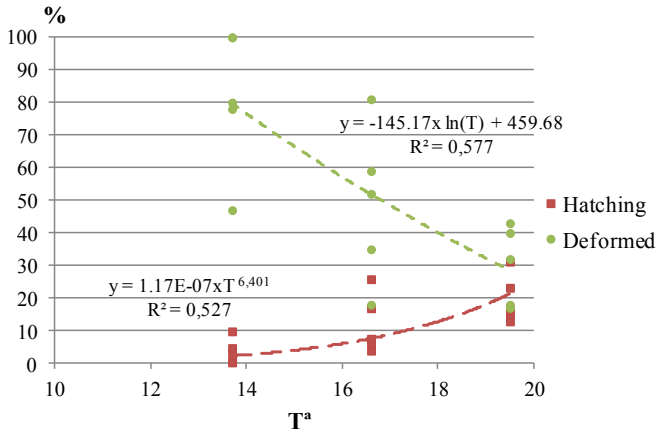


Figure 14. Regression adjustment for percent of hatched and deformed larvae as a function of incubation temperature.

The critical days regarding the viability of embryogenesis at the three temperature ranges were also calculated. **Table 2** shows the percentage of eggs collected from the bottom of each vessel during the first three days of incubation with respect to the total initial eggs stocked.

Table 2. Eggs collected from the bottom (%) the first three days of incubation at three water temperatures.

Tª	Days of incubation		
	1	2	3
13.7º C	24% ± 9.7%	30.2% ± 9.5%	29.5% ± 10.9%
16.6º C	35.4% ± 23.8%	40.8% ± 20.9%	4.7% ± 3.4%
19.5º C	56.8% ± 8%	18.2% ± 7.5%	-

At 13.7°C accumulated egg mortality was around 84% during the first three days of incubation. At 16.6ºC, the highest levels, around 76%, are concentrated in the first two days. At 19.5ºC the majority of the mortality, over 57%, was during the first day of incubation. This effect could be explained by the great vulnerability to external conditions of these eggs during the first stages of embryonic development. Although the mortality during the first day is higher at a higher temperature (19.5º C), the development of the embryos is faster and reach a more resistant state of development that greatly decreases mortality.

SECOND TRIAL OF LARVAE CULTURE WITH TWO WATER TEMPERATURES

In order to determine the optimal temperature range for wreckfish larvae husbandry, an experiment was carried out during April and May 2017 (**Fig. 15**), testing two temperatures: 15ºC-17ºC and 19ªC-21ºC, leaving aside the minimum temperature of 14ºC tested during last year 2016, due to the poor results obtained.

3 dph larvae were introduced, in triplicate, with a concentration of 7 larvae.l^{-1} in 100 l cylinder tanks, with continuous 1µm filtered water flow, slow renewal (8 $l.h^{-1}$) and soft aeration. Rotifers were enriched with $100x10^{3}$cells.ml^{-1} of Isochrysis and fed to the larvae at a concentration of 3 rot.ml^{-1}. The average temperatures obtained during the experiment were 16.4±0.6ºC and 19.7±0.6ºC for each range. Data of total length (mm) and dry weight (mg) of 10 larvae of each treatment were taken at 3 and 10 dph. The measured values are shown in the **Table 3**.

Table 3. Total length and dry weight of larvae at 3 and 10 dph cultured at different water temperature.

	16,4 ºC		19,7 ºC	
Age (days)	Length (mm)	Dry weight (mg)	Length (mm)	Dry weight (mg)
3	5.582 ± 0.364	0.3553 ± 0.0261	5.582 ± 0.364	0.3553 ± 0.0261
10	5.92 ± 0.347	0.3044 ± 0.0164	5.49 ± 0.289	0.2492 ± 0.0331

The average length increased at 10 dph in larvae reared at 16.4ºC, while it decreased in those at 19.7ºC. Dry weights also decrease in both treatments at 10 dph. This may be due to the fact that from day 8 dph at 19.7ºC a large number of larvae demonstrated a deformed mouth and operculum which prevented proper development, movement and feeding. This same effect was observed at 16.4ºC from 10 dph.

Figure 15. Cylindrical tanks for larval rearing experiments under different water temperatures.

As the larval cultures advanced, the deformities appeared in most of the larvae of both treatments causing massive mortalities. During the entire experiment no food was observed in the digestive tract. On the other hand, 1 larvae reared at 19.7ºC and 3 at 16.4ºC reached 24 and 29 dph respectively at the end of the experiment.

THIRD TRIAL OF LARVAL CULTURE WITH TWO WATER TEMPERATURES

During the last year of the project, three trials with two water temperatures of 16ºC and 19ºC were done. In the first one 1100 larvae were stocked in triplicate 400 l tanks for each water temperature resulting in a density of 10 larvae.l^{-1}. Due to a contamination problem, caused by a high hatching density, which led to poor larvae quality, at 5 dph all larval died. In the second attempt with the same conditions, larvae were alive until 12 dph.
The third trial was done in a different type of tank as well as a different larvae batch, due to a problem with the cooler system water. The culture at the lower water temperature, 15.5±0.5ºC, was made in triplicate in 100 l tanks, with a density of 10 larvae.l^{-1} whereas the experiment at 19.1±0.6ºC was made in 500 L tanks with a density of 16 larvae.l^{-1}. Samples for length and dry weight were collected at 18, 22 and 26 dph, and at 22, 24 and 26 dph for the lower and higher temperature, respectively. No statistical differences ($p>0.05$) in size or weight were observed in larvae growth at different temperatures. The mean growth (dry weight) and length during the trials are shown in **Fig. 16**.

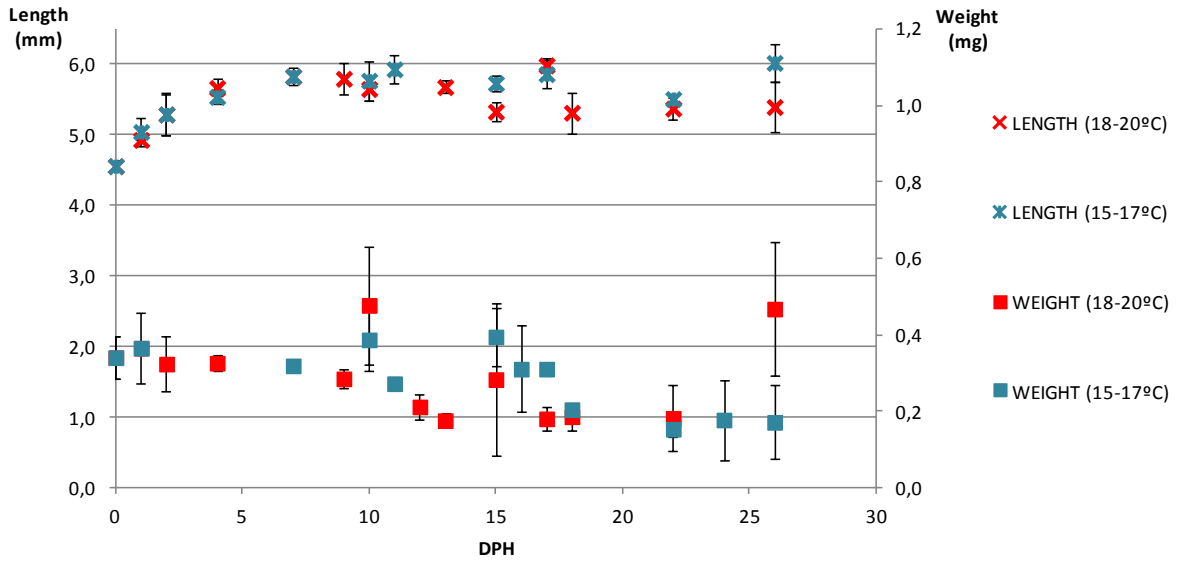


Figure 16. Length (mm) and dry weight (mg) of larvae cultured at different temperatures.

The average length increased from 4.557±0.078 (0 dph) to 6.02±0.265 (26 dph). This development occurred mainly during the first 10 days of life, staying relatively constant afterwards. Growth showed a different pattern with an irregular distribution with the larvae age. Newly hatched larvae weighed 0.3403±0.068 mg (dry weight) and the maximum values were observed in 10 dph and 26 dph fish demonstrating approximately 0.47 mg.

Conclusions and perspectives

The results so far suggest that the optimal water temperature for artificial incubation of wreckfish eggs is in a range of 16.5-19.5ºC. Lower temperatures (14±0.5ºC) promote more deformed larvae and lower hatching rates, with more egg mortalities during the first three days of incubation.
Regarding higher water incubation temperatures, it is possible to incubate at 19±0.5ºC with acceptable results in terms of deformities and hatching rates. However, due to the large size of wreckfish larvae and the density of culture tested, using very high temperatures increases the levels of ammonium, pH and opportunistic species (ciliates) that significantly alter the optimal conditions of larval husbandry. Larval rearing between 15.5±0.5ºC and 19.1±0.6ºC showed good performance of larvae up to 26 dph, mainly at the highest temperature where larvae of 0.47 mg of dry weight were obtained. The joint action of incubation between 16.5-19.5ºC and larval culture between 15.5 - 19.1ºC suggest that a temperature range between 16-18 ºC may be adequate to improve survival and growth in wreckfish. It is worth mentioning that, in the IGAFA facilities, there are 25 juveniles that reached 150 dph that were cultured at approximately 18ºC and represents a significant step forward in wreckfish larval culture and provides a basis for further studies.

References

Álvarez-Blázquez, B., Vilar, Papandroulakis, C.A, Linares, F., Cal, R., Rodríguez, J.L., Lluch, N., Gómez, C., Pérez, E., and and Peleteiro J.B. First experiences of wreckfish (Polyprion americanus) larval husbandry in NW Atlantic and East Mediterranea. Aquaculture Europe 2016 (EAS), September . Edimburg. United Kingdom.

Álvarez-Blázquez, B., Rodríguez, J.L., Vilar, A., Mylonas, C., Papandroulakis, E., Pérez Rial, E., Lluch, N., Pazos, G., Linares, F. Progress in the wreckfish intensive culture. 2017. New candidate species for aquaculture. Aquaculture Europe. October, 2017.

Ball, A.O., Sedberry, G.R., Zatcoff, M.S., Chapman, R.W., Carlin, J.L. Population structure of wreckfish *Polyprion americanus* determined with microsatellite genetic markers. 2000. Mar. Biol. 137: 1077-1090.

Kamler, E. Ontogeny of yolk-feeding fish: an ecological perspective. 2002. Rev. Fish. Biol. Fish. 12, 79-103.

Kentouri, M., Papandroulakis, N. Divanach, P. Species diversification in Greek finfish mariculture. 1995 Cah. Options Mediterr 14: 129-136.

Peleteiro, J.B., Saavedra Penas, C., Pérez Rial, E., Álvarez-Blázquez, B., Soares, E.C., Vilar Perón, A. Diversificación de especies en acuicultura. Estudio preliminar de la reproducción de la cherna (*Polyprion americanus*) en cautividad., Congreso Nacional de Acuicultura, 2011 Castelldefels, Spain.

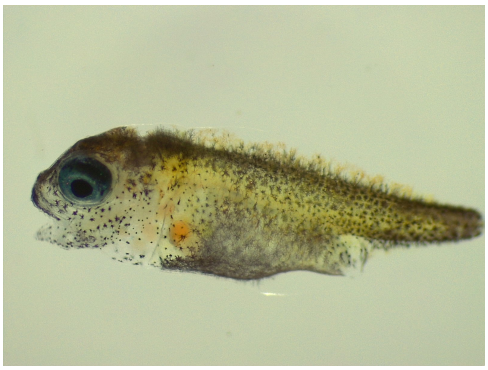
Rodriguez-Villanueva J.L., Peleteiro J.B., Perez-Rial E., Soares E.C., Álvarez-Blázquez B., Mariño C., Linares F., Mañanós E. Growth of wreckfish (Polyprion americanus) in Galicia, Spain. 2011. Aquaculture Europe.

Sedberry, G.R., Andrade, C.A.P., Carlin, J.L., Chapman, R.W., Luckhurst, B.E., Manooch, C.S. III, Menezes, G., Thomsen, B., Ulrich, G.F. Wreckfish *Polyprion americanus* in the North Atlantic: fisheries, biology and management of a widely distributed and long-lived fish. 1999. American

Fisheries Society Symposium. Life in slow lane: ecology and conservation of long lived marine animals, nº23, pp. 27-50

Papandroulakis N, Divanach P and Kentouri M. Specific diversification in finfish mariculture: the Mediterranean case. Proceeding of the International Conference Martinique Special Publication. European Aquaculture 199, pp 223-224.

Papandroulakis N, M. Suquet, M.T. Spedicato, A.Machias, C. Fauvel and P Divanach. Feeding rates, growth performance and gametogénesis of wreckfish (Polyprion americanus) kept in captivity. 2004. Aquaculture International 12: 395-40



Co-funded by the Seventh Framework Programme of the European Union