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Research Article

Suitability of artisanal fishery discards as feed for juvenile tub gurnard (*Chelidonichthys lucerna L.*) reared in sea bottom cages in the mid Adriatic Sea

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Abstract

The suitability of using discards from artisanal fisheries as feed for wild-caught *Chelidonichthys lucerna* (L.) reared in submerged cages in the Adriatic Sea was investigated. Three-hundred juvenile tub gurnard (72.9 ± 11 g, 18 ± 1 cm) were captured and separated into four circular 35.3 m³ submerged cages. Two stocking densities were used: two cages contained 60 fish each (1.7 fish m⁻³; CG-L) and two cages contained 90 fish each (2.55 fish m⁻³; CG-H). Growth performance and survival rates were recorded over a 240 day period during which tub gurnard were fed with a variety non-target fishery discards, including common crab, sardine, Atlantic chub mackerel, anchovy, and salema. The survival rate was approximately 90% in both groups (CG-L = 91% and CG-H = 90%), with final stocking densities of 0.32 kg m⁻³ and 0.49 kg m⁻³ for the CG-L and CG-H groups, respectively. There were no significant differences in final mean body weight or length between the CG-L group (206 ± 23 g, 24.6 ± 2 cm) and the CG-H group (215.5 ± 32 g, 24.8 ± 2 cm). The condition index was similar between the two groups (1.38 and 1.42 for CG-L and CG-H, respectively). The feed conversion ratio was high in both the CG-H (2.39) and CG-L (2.32) cages. These results demonstrate that viable growth rates of tub gurnard may be obtained by feeding recycled fishery discards and rearing in cages placed on the seabed, which allows for the natural benthic behavior of this species.

Keywords: Tub gurnard, bottom cage aquaculture, feeding, fishery discards, growth, survival rate.

Introduction

Over the last ten years, professional fishing within the European Union (EU) has incurred several major problems, leading to lower profitability and the loss of employment. The problems include increased production costs, greater efforts to preserve fish stocks and biological resources, and increased fish imports from a highly competitive international market. Employment in the fisheries industry experienced a decline of 31% between 2002 and 2010, according to the European Commission. Coastal areas represent around 54% of total fishery employment and, therefore, this issue is of particular concern in these places (Natale *et al*., 2013). A significant reduction in fishery fleet sizes and landings, as well as increases in fishery product imports, have been reported at the Italian level.

Due to these various issues, fishermen are diversifying their strategies to develop markets toward a sustainable fishery. For instance, the Marche Region of Italy, with 174 km of coast, is a traditional fishing area characterized by shellfish, anchovy, clam, and cephalopod fisheries. The fishermen in this region have become more focused on the fish and fish processing industry, capturing mostly demersal species, such as hake, flatfish, and tub gurnard.

Tub gurnard (*Chelidonichthys lucerna* L.) is of great socio-economic importance to the coastal communities of the mid Adriatic Sea. This species has facilitated the recovery and enhancement of traditional activities, in addition to new initiatives such as fish "Zero kilometer", which provides a high quality product at a fair price to consumers, promotes the daily consumption of fish, and thus enhances the excellence of local fish production. Demand for this species is continuously increasing in this region of Italy as there is a strong tradition of consuming this species and it is appreciated for its lean meat (Roncarati *et al*., 2014). Previous studies (D'Andrea *et al*., 2012; Roncarati *et al*., 2013) evaluated the potential domestication of *C. lucerna*, focusing on how this species responds to aquaculture practices (Parisi *et al*., 2014). One study also noted that tub gurnard exhibit opportunistic foraging behavior, mainly preying on epibenthic and nectobenthic organisms (Stagioni *et al*., 2012) that are highly represented in fishery discards.

Artisanal fishery practices in the mid Adriatic Sea consist of nets positioned on the seabed at sunset and then removed at sunrise, with the aim of capturing target fish (e.g., hake and gurnard); however, non-target species (bycatch) are also attracted to the nets and are incidentally captured. Trawling and coastal fishing with trammel nets generate large quantities of discards (i.e., unwanted bycatch), mainly consisting of invertebrates and unmarketable fish (Brill *et al*., 2008). The EU Common Fisheries Policy (CFP) is focused on finding ways to prevent wasting such discards. Because these discarded fish represent an important source of protein and lipid, one

promising solution is to recycle artisanal fishery discards as feed for aquaculture species such as tub gurnard. Most tub gurnard captured for market tend to be small (less than 150 g); however, larger sized fish (which represent a small percentage of wild capture) command greater market prices. Thus, capturing and rearing undersized tub gurnard on fishery discards represents a potentially profitable strategy.

This study investigated the suitability of artisanal fishery discards for feeding juvenile tub gurnard reared in cylindrical bottom cages (classified as benthic cages). A feeding trial was performed to evaluate the growth performance and survival of tub gurnard fed fishery discards for 240 days. Production performance was determined by evaluating the final weight and size of fish stocked at different densities. The results of the present study are expected to provide evidence to support the use of this strategy to rear tub gurnard of high economic value.

Materials and Methods

Experimental cages

The trial was conducted near a long-line mussel farm, along the coast of Porto San Giorgio, Italy. The site was located 2.3 to 2.9 miles from shore, at a depth of 13 m. The site was characterized by a low sea current with an average speed of 0.5-1 cm s^{-1}.

In September 2014, four cage modules were constructed and sunk to the seabed. The cages were net-pens with a height of 1.8 m constructed from 2 circular frames (diameter = 5 m) made of high density polyethylene (HDPE) pipes (tube diameter = 75 mm) separated by 7 HDPE vertical pipes (tube diameter = 75 mm) inserted with appropriate galvanized metal connectors. Each structure supported a 25-mm mesh nylon net bag with a volume 35.3 m^3.

The cages were lowered to the sea floor using a crane on the deck of the tug. Openings in the HDPE tubing allowed for flooding of the cage structure, causing it to sink. Each cage was anchored to the sea bottom by 4 parallel concrete blocks (1 x 1 x 0.4 m) weighing 700 kg each. The blocks were placed on the seabed before sinking the cage structure. The lower base of the cage was affixed to the blocks using high-strength galvanized chains (3 cm diameter and 1.5 m length), permanently anchoring the structure to the seabed. The blocks secured the structure to the seabed at four points, in line with the axes of the bases of the cylinder.

Fish used for the rearing trials

The *C. lucerna* juveniles (mean body weight 72.9 ± 11 g, mean length 18 ± 1 cm) used in the trial were selected from specimens captured with hooks during 8 fishing sessions in September 2014, a time when wild-captured individuals of this species tend to be small. On board the fishing vessel, live fish were held in two 150 L tanks with aeration and a seawater exchange pump. Each fish was weighed to the nearest 0.1 g fresh weight with an electronic balance (Mettler 5000, Mettler Toledo International, Novate Milanese, Italy) and the total body length (from the most anterior extremity to the caudal fin) was measured to the nearest millimeter using an ictiometer. Of the total fish captured (n = 520), 25% died following capture or during the following three days and 17% of the remaining fish were excluded due to high variation in size compared to the average body weight.

Three-hundred juveniles were used in the trial. The fish were separated into the four cages at two different stocking densities: 60 fish per cage (1.7 fish m^{-3}) in two cages (CG-L) and 90 fish per cage (2.55 fish m^{-3}) in the other two cages (CG-H) (Table 1).

Discard feeding and supply system

Different types of food were used to feed the juveniles. The diet was 46% crustacean (44% common crab, *Carcinus aestuarii* [Nardo] and 2% mantis shrimp, *Squilla mantis* [Fabricius]) and 54% fish (35% Atlantic chub mackerel, *Scomber colias* [L.], 15% salema, *Salpa salpa* [L.], and 4% sprat, *Sprattus sprattus* [L.]). The chemical composition of the diet (crustaceans and fish) was determined prior to the beginning of the feeding trial (Table 2). The organisms were pooled, homogenized, and submitted to proximate analysis (moisture, protein, lipid,

Table 1. Main parameters of the feeding trial (CG-L = low-density cages; CG-H = high-density cages).

	CG-L	CG-H
Replicates (cages per group)	2	2
Initial weight (g) (mean ± SD) (n = 300)	72.9 ± 11	72.9 ± 11
Initial mean length (cm) (mean ± SD)	18 ± 1	18 ± 1
Initial stocking density (fish m^{-3})	1.7	2.55
Initial biomass (kg cage)	4.38	6.56
Fattening period (days)	240	240

Table 2. Chemical composition and categories of fatty acids in the mixed organisms (crustaceans and fish) administered as feed to tub gurnard reared in the two groups of bottom cages. (Averages are means of three replicates).

Chemical Composition (%)	
Moisture	77.5 ± 3.1
Protein	20.03 ± 1.9
Lipid	1.81 ± 1.2
Ash	1.51 ± 0.2
Fatty Acid Categories (% of total fatty acids)	
Saturated	31.01 ± 2.2
Monounsaturated	25.26 ± 5.9
Polyunsaturated	37.62 ± 7.2
Total n-3	31.99 ± 8.0
Total n-6	5.64 ± 1.1

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and ash content) in triplicate. The percentage of moisture and ash content were determined according to the Association of Official Analytical Chemists procedure (AOAC, 1990). Protein content was determined using the standard Kjeldahl copper catalyst method (AOAC, 1990). Total lipids were measured using a modification of the chloroform:methanol procedure described by Folch *et al.* (1957). After determining total lipid content, fatty acids were converted to methyl esters following the method described by Christopherson & Glass (1969). The separation of fatty acids was carried out by using a GC 3800 gas chromatograph (Varian Strumentazione, Cernusco sul Naviglio, Italy) with a WP-4 Shimadzu integration system (Shimadzu Corporation, Tokyo, Japan), which was equipped with a Supelco SP^{TM} – 2340 capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness; Supelco, Bellefonte, Pennsylvania, USA) and a flame ionization detector.

Fish and crustaceans were supplied via a feeding pipe composed of a flexible plastic corrugated tube that was kept at the top of the cage by a float. The feeding pipe had a stainless steel core (diameter = 60 mm), was 14 m long, and was semitransparent to visually check that the food passed through without blockage. The speed of food passage was regulated by a water pump, with food and water descending at a speed of 0.7-1 m sec^{-1}.

Fish were fed to satiation at around 09:00 five days per week. The initial daily food ration represented 8% of fish biomass. At 60, 120, and 180 days, the food ration was reduced to 7%, 6%, and 5% of fish biomass, respectively, based on fish body weight and mortality rate. Uneaten food and dead fish in the cage were checked visually by a professional diver from the fishermen cooperative, who entered through an opening (0.7 x 0.7 m) at the top of each cage and removed all items in order to maintain the quality of the rearing environment.

The temperature and dissolved oxygen in the water were measured directly with a portable electronic device (mod. 55 YSI, Yellow Springs, Ohio, USA). Salinity was measured with a hand-held refractometer (Master 53-S ATAGO CO. Ltd, Tokyo, Japan). Nitrogen parameters (ammonia nitrogen and nitrate) were analyzed using a spectrophotometer (DR 5000, HACH Company, Loveland, USA), following the international methods of the American Public Health Association (APHA, 1989).

Sampling and monitoring activities

In accordance with the availability of diver operators and the suitability of weather conditions, the cages were lifted out of the water monthly for cleaning of the nets to maintain consistent flow through the cages.

Every 30 days, 10 fish from each cage were separately weighed and measured to evaluate and adjust the quantity of feed. At the end of the trial, the main biomorphometric parameters were recorded and used to calculate the condition index (KI = [fish weight / fish length^3]

x 100). Fish growth rate was calculated as the specific growth rate (SGR) (Ricker, 1979) using the formula: SGR (%) = (ln Wf – ln Wi) x 100 / t, where ln Wt and ln Wi are the natural logarithms of the final mean weight and the initial mean weight, respectively, after t days.

Survival was determined by recording the number of live individuals in each cage. Survival was expressed as a percentage according to the formula: S (%) = Ma / Ni x 100, where Ma is the accumulated mortality and Ni is the initial number of animals (Ricker, 1979).

Somatic indices were determined by the viscerosomatic index (VSI = [visceral weight / whole-body weight] x 100) and the hepatosomatic index (HSI = [liver weight / body weight] x 100) (Roncarati *et al.*, 2010). Fish were sacrificed in water and ice, then gastric contents were removed and prey items were identified and sorted into taxonomic groups when possible. The final stocking density (kg m^{-3}), survival rate (%), and food conversion rate (FCR) were also evaluated by calculating the ratio between the total weight of food given and the total weight of fish harvested.

Liver histology and analysis

At the end of the experiment, the livers of five gurnards from each cage were fixed in 10% buffered formalin. Standard histological techniques were applied to investigate potential differences in hepatocyte condition between cage-reared and wild gurnards of similar size captured in the same area. A representative portion of each sample was dehydrated in ethanol, clarified in xylene, and embedded in paraffin wax. Tissue was serially sectioned at 4 μm using a rotary microtome (Leica RM2235, Leica Microsystems, Wetzlar, Germany). The slides were stained with hematoxylin and eosin. Processing of the tissues took place at the Laboratory of Animal Pathology in the Department of Veterinary Medicine, Camerino University, Italy. Histological examination was performed by light microscopy (Nikon Phase Contrast 0.90 Dry, Japan). Tissue morphology was assessed according to a grading system (absent, light, and high damage) used to observe possible changes in hepatic cell morphology. In addition, the integrity of the fins was used to evaluate the condition of all subjects from all of the cages, as an indicator of well-being and as a measure of the quality of farmed fish.

Statistics

Analysis of variance (ANOVA) using the SAS General Model procedure was conducted to detect differences between the two groups with respect to the final results of growth performance and other indices. Statistical differences were identified using the Student Newman-Keuls test (SAS Institute, 1988). Differences were considered significant at P < 0.05.

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Results

The final productive performance and indices of the juvenile tub gurnard are presented in Table 3. There was no significant difference in the mean body weight between the CG-L and CG-H cages; 206 ± 23 g and 215.5 ± 32 g, respectively. Body length was also similar in both groups; 24.6 ± 2 cm (CG-L) and 24.8 ± 2 cm (CG-L). There was no difference in the condition index between the two groups; 0.85 (CG-L) and 0.82 (CG-L).

The final stocking density was 0.32 kg m^{-3} and 0.49 kg m^{-3} in the CG-H and CG-L groups, respectively. No significant difference in HSI was observed between the CG-H (HSI = 2.5) and CG-L (HSI = 2.34) groups. Similarly, no significant difference was observed in the VSI (5.03 and 5.99 in the CG-H and CG-L groups, respectively). A similar specific growth was observed in the two groups; 0.43 (CG-L) and 0.45 (CG-H). Gastric contents revealed the presence of all species administered as feed with crustaceans showing the highest incidence in both the groups, followed by Atlantic chub mackerel and the other two fish species. The survival rate was similar in both groups (CG-L = 91%; CG-H = 90%). The feed conversion ratio was high in both groups (2.39 and 2.32 for CG-L and CG-H, respectively).

Water temperature varied in relation to fish weight sampling times (Fig. 1). Water temperature remained above 15°C from October to the end of December, and above 20°C from the second half of May to June. The DO level and salinity were at their lowest in the second week of October and the first days of November (7.8 and 30.5 mg L^{-1}, respectively) and were highest in June (18.5 and 33.8 mg L^{-1}, respectively). The pH was at its lowest in October (7.95) and highest in March (8.25).

The histological analysis confirmed the absence of significant hepatic alterations in the reared tub gurnard.

Table 3. Biomorphometric parameters, somatic indices, and production values at the end of the trial (CG-L = low-density cages; CG-H = high-density cages; HSI = hepatosomatic index; KI = condition index; VSI = viscerosomatic index).

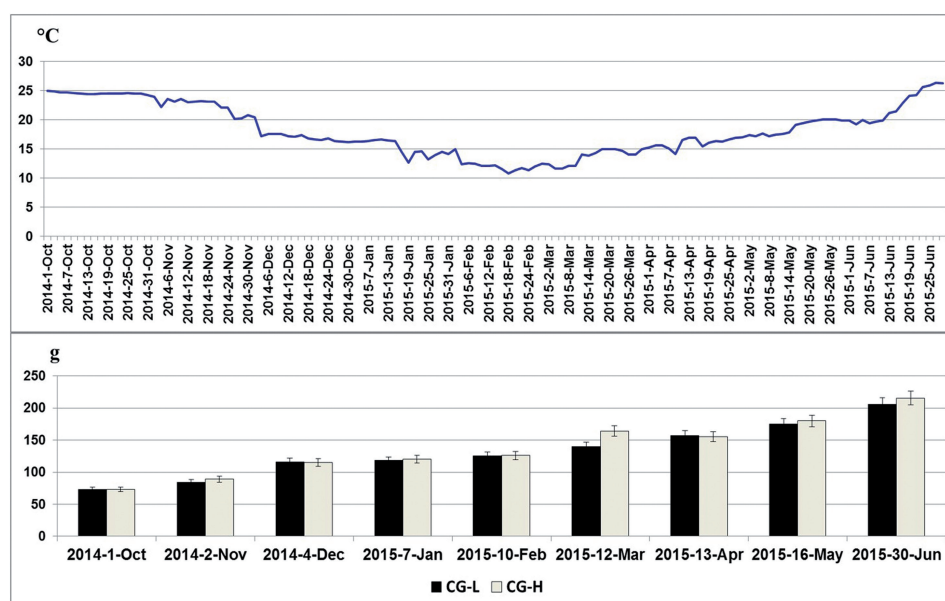| Parameter	Units	CG-L	CG-H
Final body weight	g	206 ± 23	215.5 ± 32
Final total length	cm	24.6 ± 2	24.8 ± 2
KI	-	1.38 ± 0.8	1.41 ± 0.7
HSI	-	2.5 ± 0.6	2.34 ± 0.5
VSI	-	5.03 ± 0.5	5.99 ± 0.6
Specific growth rate	%	0.43 ± 0.4	0.45 ± 0.6
Survival rate	%	91 ± 1.4	90 ± 1
Food conversion rate	kg/kg	2.39 ± 0.5	2.32 ± 0.4
Final density	kg m^{-3}	0.32 ± 0.7	0.49 ± 0.8

Fig. 1: Water temperature (upper chart) and mean body weight of tub gurnard reared in bottom cages (lower chart; data presented as mean ± SD) during the feeding trial.

Modest steatosis (grade I) was detected in all reared gurnards, but not in wild subjects captured at the site of the experiment (Fig. 2,3). The reared fish tissue appeared to be well differentiated, with rich melano-macrophage centers and cells with intact nuclei, confirming the absence of important degenerative states.

Discussion

This study has demonstrated that artisanal fishery by-catch discards can be used to feed small-sized juvenile tub gurnards reared in seabed cages, resulting in fish of good condition. These cage-reared tub gurnard should be readily accepted at market as there is a considerable local demand for this species.

Compared to the stocking densities used for the intensive rearing of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (both euryhaline species), or other marine finfish farmed in cages (>9 kg m^{-3}), the two stocking densities used in this study were low. The densities selected in this study were only tested in two replicate cages per group due to financial constraints. Nevertheless, these stocking densities provided important information about the feasibility of rearing tub gurnard in bottom cages. Sea bream and sea bass exhibit high growth rates at high stocking densities (≤25 kg m^{-3}) in submerged cage modules ((Kavadias *et al*., 2003; Roncarati *et al.,* 2010; Maricchiolo *et al*., 2011). In contrast, warm tropical species, such as cobia (*Rachycentron canadum)* and mahi-mahi (*Coryphaena hippurus*), exhibit high growth rates at lower densities (≤10 kg m^{-3}) (Benetti *et al*., 2010).

The stocking densities used in the current study were very low and may have been responsible for the high survival rate (>90%). The benthic position of the cages may have also improved survival during the feeding trial. The cage structure was stable with low net deformation, which is considered to enhance the growth of caged fish

(Lader *et al*., 2008). A reduction in net volume has been shown to have a negative effect on welfare and increase disturbance of Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*) (Kristiansen *et al*., 2004). Environmental parameters may also affect the health of farmed fish. The health of benthic organisms is directly related to the dispersion of waste by water currents for both inshore and offshore cages (Forchino *et al*., 2011). In the current study, water quality remained stable and appeared to be sufficient to maintain fish health. To generate suitable environments for fish growth, the correct design, mooring system, hydrodynamics, and volume are required (Huang *et al*., 2007; Shainee *et al*., 2013). The present study ensured that a high concentration of dissolved oxygen was present in the fish cages because this parameter influences the growth and survival rate of cultured fish (Roncarati *et al.,* 2013; Hwang *et al.*, 2014).

In the Mediterranean Sea, tub gurnard naturally occurs at depths of 10 to 150 m, where temperatures usually remain below 20°C, even during the summer. The results of the current study indicate that tub gurnard growth rates were positively influenced by increasing water temperature. The best growth rates were detected from October to November at temperatures between 20°C and 25°C. Lower growth rates were obtained from December to March when the water temperature did not exceed 15°C. In comparison, high mortality of gurnards was detected in basins where the sea temperature exceeded 25°C (Roncarati *et al*., 2013). These various studies demonstrate the importance of rearing gurnard under optimal conditions.

In the current study, tub gurnard did not exhibit any territorial or cannibalistic behavior, which tend to be evident during the juvenile phase, disappearing with growth and in the presence of sufficient feed resources to satisfy metabolic needs. The adopted feeding plan was favorable because it largely reflected the feeding habits of gurnard in the local environment. Many studies have shown that fishery discards, which are not targeted by the
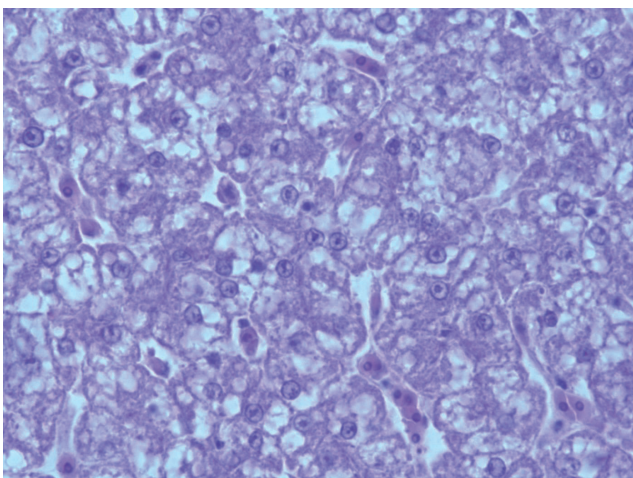

Fig. 2: Histological examination of the liver from wild tub gurnard (hematoxylin and eosin stain; 40x magnification).
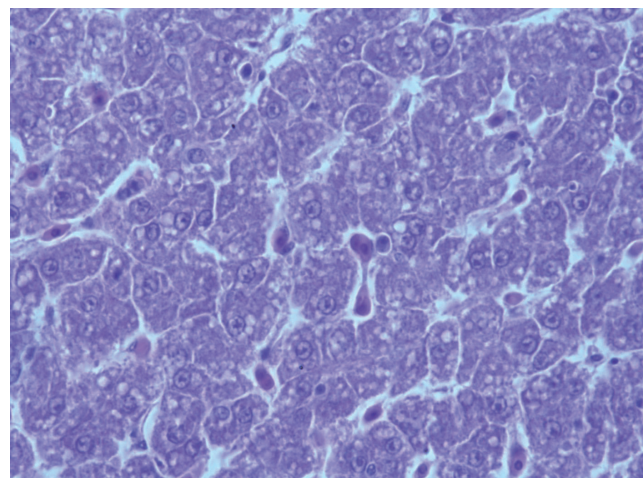

Fig. 3: Histological examination of the liver from cage-reared tub gurnard (hematoxylin and eosin stain; 40x magnification).

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fishery, may affect the biodiversity of the marine ecosystem (Johnsen & Eliasen, 2011; Heath *et al*., 2014; Sardà *et al*., 2015). When the current study was granted, the new EU CFP had not yet come into force; however, this study confirms that local fish species that are excluded by the market contain high amounts of protein and n-3-rich fatty acids. Thus, artisanal fishery discards should be considered as a good value-added diet for feeding aquatic organisms (Ordoñez-Del Pazo *et al*., 2014). The administration of a diet consisting entirely of fishery discards resulted in a good condition index of the cage-reared tub gurnard. This result was confirmed by gut content analysis, with several specimens having fed on crabs of similar size that were appropriate for their current stage of the growing cycle. Crustaceans and fish are rich in important nutrients, such as essential amino acids and omega 3 fatty acids (King *et al*., 1990; Bono *et al*., 2012), and were sufficient for the maintenance and growth of tub gurnard under farmed conditions in this study. The FCR appeared to be high but it is necessary to consider that the fresh weight of food items with high moisture content was used in the calculation of the FCR.

Despite being of considerable importance to the aquaculture industry, research into the development of technologies for rearing fish in bottom cages is still in the preliminary stage (Doxa *et al*., 2011). The development of bottom cage technology may help resolve the current issues that exist with rearing tub gurnard and other benthic marine species that would be considered potentially interesting to aquaculture. For instance, bottom cages reduce the discoloration of red porgy (*Pagrus pagrus* L.) due to low light penetration (Papandroulakis *et al*., 2013). Bottom cages produce satisfactory growth rates for lobster (*Homarus gammarus*) (James, 2007). Bottom cages also enhance the growth of octopus (*Octopus vulgaris*) compared to floating-type cages (Estefanell *et al*., 2012).

The results of this study demonstrate that it is possible to create alternative fisheries techniques using fishery discards to optimize the farming of an economically important benthic fish species. In this case, the economically important species was the tub gurnard, a species native to the Marche Region coast. It was confirmed that low-cost structures could be used as bottom cages, potentially creating new aquaculture opportunities that require small investments and utilize the marine environment. This project has demonstrated the technical reliability of equipment and economic feasibility of an innovative technology to exploit artisanal fishery discards in the Adriatic Sea.

This study presented a strategy to enhance seafood production, in particular local fish production. Small tub gurnard tend to be captured in the local area, whereas larger fish are preferred by consumers. By placing this species in aquaculture conditions and feeding it with artisanal fishery discards, the market value of the fish could be significantly enhanced after less than one year. Thus, this fish presents an interesting aquaculture species that would help fisheries meet EU CFP regulations (by not discarding bycatch) as they shift toward aquaculture activities. This study also presents a preliminary design for sea bottom cages that enhance fish zootechnical performance. The use of discards significantly reduces the cost of fish production while the fish receive the high value nutrients that they would preferentially consume in the wild. This study confirms that wild tub gurnard juveniles can be reared under captive conditions (in sea bottom cages) on fishery discards while attaining good rates of growth and survival.

Acknowledgements

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