

Final Report

Developing an effective release-method for restocking hatchery-bred Brown-marbled grouper (*Epinephelus fuscoguttatus*)

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Summary

There is renewed interest in stocking programmes, as developments in aquaculture can produce high amounts of fish juveniles at low costs, e.g. groupers. They are commercially interesting species, but their stocks are poorly monitored. To bolster their populations they are a good focus for stocking programmes. However, there is still a lot of debate going on about the survival rate and impacts of hatchery-bred fish that are released in the wild in general. This research therefore studied the behaviour of hatchery-bred brown-marbled groupers (*Epinephelus fuscoguttatus*) in the presence of a predator and how the fingerlings dealt with their new environment right after release. A difference in behaviour was indeed observed between groupers that were exposed to predation and a control group, but after a week the differences became less, probably due to habituation. Training them in constant presence of a predator is therefore discouraged for prolonged time periods.

Groupers would familiarize themselves with their environment within a day after release, but this is assumed to be dependent on the density of released individuals. Low densities prevent fingerlings to resort to defensive strategies, such as schooling behaviour. Higher densities are assumed to induce the competition between released conspecifics, lowering the survivability post-release. Based on these findings it is concluded that a stocking programme of brown-marbled groupers can be successful if conducted efficiently and after careful examination of the possibilities. It is elementary that the quality of the ecosystem is sufficient to support a self-replenishing population of brown-marbled groupers. Besides, the stocking of a top predator, such as the brown-marbled grouper, can easily restructure the fish community in the area and this should therefore be regularly monitored.

Introduction

The projections are that many fish stocks all over the world will become depleted in a fast rate (Pauly, *et al.*, 2002). Overexploitation, habitat loss, and the introduction of invasive species are the main agents responsible for the fast decline of fish populations over the past decades (Lipcius, *et al.*, 2008; Coleman, *et al.*, 2000; Brown & Laland, 2001; Brokordt, *et al.*, 2006). The impacts of anthropological activities on marine life are relatively difficult to assess. On contrary to the clear impacts humans have on the landscape above the water surface, relatively few people can witness the impacts below the surface. Still, the effects of human actions are becoming more profound as exploitation is rapidly reducing the abundance and distribution of many marine fish species (Lipcius, *et al.*, 2008), which also starts to become apparent in the steady decline of fishery catches all over the world (FAO, 2000). However, demands for fish production is projected to keep increasing by 1.5% annually (Delgado, *et al.*, 2003), and this will have further negative impacts on fish populations (Lipcius, *et al.*, 2008; Coleman, *et al.*, 2000). Fishing effort needs to decrease drastically if fisheries want to be sustainable in the long-term and want stable landings in decades to come.

This is unlikely going to happen and aquaculture will therefore play a major role in supplying the high demands for fish (Bell, *et al.*, 2006). To support wild fish populations in sustaining the high fishing pressure, many stocks are bolstered by releasing fingerlings that are bred and nursed in hatcheries (Brown & Laland, 2001; La Mesa, *et al.*, 2008). There has been a renewed interest in stocking reared fish in order to enhance natural populations for conservation purposes and to restore depleted stocks of endangered species (Vilhunén, 2006; Brown & Day, 2002; Delgado, *et al.*, 2003).

However, despite many previous restocking attempts with several marine fish species there is little data supporting that releases of hatchery-bred fish actually enhances natural populations (Salvanes & Braithwaite, 2006; Brown & Laland, 2001). Fish-rearing often uses commercial aquaculture techniques, which focus on growth, survival, and reproduction and it ignores the importance of behavioural traits required for living in the wild (Vilhunén, 2006). This causes a relative high mortality of the hatchery-reared fish after release, resulting in a high failure rate of restocking programmes (Brown & Laland, 2001; Vilhunén, 2006).

The main source of this high post-release mortality of hatchery-reared fish is still under debate. According to Brown and Day (2002), malnutrition is the main reason for this high mortality. This is supported by La Mesa, *et al.* (2008), who address the competition between conspecifics due to limited feeding and habitat resources as a major contributor to the low survival rate. However, Brown and Laland (2001) state that the high mortality in released fish is mainly due to impaired predator-avoidance behaviour and predator 'naiveness'. The vulnerability for diseases might also play an important role in the poor survivability of hatchery-reared fish post-release (Brokordt, Fernández, & Gaymer, 2006).

Even though survivability of the released individuals is low, most countries in the world are participating in the conduction of stocking programmes. The largest stocking programmes involve species such as salmon (*Oncorhynchus* and *Salmo* spp.), sturgeon (*Acipenser* spp.) and flounder (*Paralichthys* spp.) (Brown & Laland, 2001), and they tend to be relatively successful. In order to rebuild the spawning stock biomass, it will still require: 1) a good cooperation between capture fisheries and aquaculture, 2) a reduction in fishing capacity and effort, and 3) a restoration of the natural habitats (Pauly, *et al.*, 2002; Bell, *et al.*, 2008). However, due to newly developed methods in breeding and rearing fish at lower cost, there is renewed interest in restocking endangered and overexploited fish species for conservation purposes (Vilhunén, 2006; Bell, *et al.*, 2008). It is shown that the recovery time in cod (*Gadus morhua*) after a stressor was applied, can be reduced by growing them tanks with a variable environment (Braithwaite & Salvanes, 2005). Besides, rearing them in these enriched tanks reduces the time required to train them to feed on live prey and makes them more bold towards new experiences. This can be disadvantageous, for example when boldness is displayed in front of a predator. Still, it turns out that training them in a variable environment will also make them faster in seeking shelter (Braithwaite & Salvanes, 2005).

Such nursing methods are starting to become prevalent in research experiments studying the low post-release survival (Vilhunen, 2006). However, stock enhancement programmes are still mainly focusing on releasing large quantities of fish in the hope some will survive (Braithwaite & Salvanes, 2005), even though training for foraging skills and anti-predator behaviour is no longer expensive, and is relatively simple to conduct. The emphasis should therefore be shifted from quantity to quality in order to improve hatchery efficiency (Brown & Laland, 2001), if the objective is to bolster wild populations (Vilhunen, 2006).

Even if post-release survival is high, there are still other issues that need to be considered when stocking as a conservation method. Many authors warn for weakening of the genetic structure of the wild population in case of stock enhancement programmes (Salvanes & Braithwaite, 2006; Brown & Day, 2002; La Mesa, *et al.*, 2008). Introduction of large quantities of fingerlings from the same batch can result in a mixing of the domestic and wild fish. This leads to a reduction of the genetic variability of the natural population, and consequently will limit the possibility for future adaptations to environmental variabilities (Madeira, *et al.*, 2005). Besides, it is generally assumed that the domestication process puts a selection pressure on aggressive fish with exaggerated competitive behaviour. If these domesticated fish interact with their wild counterparts, they might outcompete their natural conspecifics (Salvanes & Braithwaite, 2006). These consequences can put an additional pressure on local fish populations, and might even drive endangered species to extinction (Braithwaite & Salvanes, 2005).

There is also serious concern about the impacts of releasing large quantities of hatchery-bred fish on the rest of the ecosystem and vice versa (Bell, *et al.*, 2006). Successful restocking as a fisheries management tool can have large impacts on the ecosystem and might even induce shifts to alternative stable states (La Mesa, *et al.*, 2008). Probably the most well-known example is the release of Nile perch (*Lates niloticus*) in Lake Victoria, which lead to a mass extinction of hundreds of native species. However, ecosystems can also impose constraints on the released fish, especially when carrying capacity is low (La Mesa, *et al.*, 2008). Releasing large amounts of fish for stocking purposes is not very efficient if conspecific competition is the main source of their mortality (Pauly, *et al.*, 2002). Another reason ecosystems can have large impacts is when the natural habitat is degraded. Restocking projects are futile, if the fish species cannot complete their life cycle in the environment and hence will not contribute to the spawning biomass (Bell, *et al.*, 2008).

Grouper background information

With an estimated 32 percent of the world fish stocks being overexploited, depleted, or recovering (FAO, 2000), it is hard to define a clear focus which species require priority in stock enhancement programmes. However, species higher in the foodweb often have more impact on the rest of the ecosystem compared to species lower in the food chain. Especially when the ecosystem is complex, contains a lot of interactions between several species, and has a high value for the local environment. Reef structures are good examples of such high-valued, complex ecosystems. Disruptions of these ecosystems can lead to infestations and disappearance of coral structures or fish species. Maintaining and restoring reef fish populations is essential in order to conserve coral reefs in a sustainable way (Coleman, *et al.*, 2000). However, many fish species that are of high economic value have life history traits that make them less resilient to high fishing pressures. They are often slow growing and reproduce at old age, aggregate at specific locations for spawning, and are protogynous hermaphrodite (switching from female to male at an older age) (Coleman, *et al.*, 2000). Fishermen often target older and larger individuals, but this can have severe consequences. Besides the concept: 'fishing down the food chain', there is also a risk to disrupt the sex ratio within a species by catching all the males (Coleman, *et al.*, 2000), or to catch away the big, older females that are highly fertile (Arkive, 2012). This can have large impacts on the fidelity, since not enough eggs are fertilized due to the lower proportion of males, or spawning of eggs is insufficient due to the disappearance of large females, or both. Most grouper species and snapper species are typical examples of reef fishes that are particularly prone to overexploitation (Coleman, *et al.*, 2000).

Groupers (Serranidae) and snappers (Lutjanidae) are economically important fish species throughout the world, and especially in Asia, because of their high market value and desirable taste (Chiu, *et al.*, 2008). Even in large densities their survival rate is relatively high, making them very suitable for grow-out purposes (Chiu, *et al.*, 2008). In Puerto Princesa, on the Island of Palawan (Philippines), Brown-marbled groupers (*Epinephelus fuscoguttatus*) are reared for consumption purposes. Fingerlings are bred in hatcheries by the Centre for Sustainability (CS) and a proportion is sold to the local population for ranching. The groupers are eventually sold on the market or exported to other countries in Asia. Brown-marbled grouper is labelled as near threatened by IUCN, because they are poorly monitored and vulnerable to overfishing (Cornish, 2012). They are classic example of a long-lived reef fish that is especially susceptible to overexploitation. They form large aggregations every month in order to spawn, making them an easy target for fishermen. They are long-living, over 40 years, and they reproduce at a relative old age, thus it will take a long time period before they recover if their stock becomes depleted. Brown-marbled groupers are protogynous hermaphrodite, and maintaining a healthy sex ratio is essential for a sustainable and healthy stock (Arkive, 2012). Status about the brown-marbled grouper stocks are unknown (Cornish, 2012), which makes quantifying the health of their stocks an issue.

This report addresses the requirements needed in order to stock Brown-marbled groupers in the Abucayan inlet of Puerto Princesa bay. To the best of my knowledge, no articles are published on restocking or stock enhancement programmes of Brown-marbled grouper. Many other grouper species are part of restocking programmes (Coleman, *et al.*, 2000; La Mesa, *et al.*, 2008; Bell, 1999; Lipcius, *et al.*, 2008), but data on stocking and recapture experiments are often insufficient and incomplete. Conducting a good monitoring shortly after release of the fish remains a challenge after all (Vilhunen, 2006). Henderson (1980) was able to describe the recovery of normal behaviour by released fish. He suggests that this happens in a sequence of three steps: 1) a short period where the fish typically display protective strategies, such as freezing, hiding, and schooling behaviour, 2) a period where the fish have to familiarize themselves with the new environment, and 3) a period where they are regaining their normal feeding strategies. In total, Vilhunen (2006) suggest that the whole familiarization process of the released fish takes approximately three days.

Materials & Methods

Groupers were reared in white tanks with a diameter of approximately 2.5 metres and a water depth of approximately 30 cm. They were handfed with pellets six times a day, and the tanks were cleaned every day. If they displayed cannibalization, they were caught and separated from each other. If the eaten individual showed signs of weakness, it was placed in a separate aquarium where it could recover. There was a small constant inflow of seawater into the tanks. Groupers were raised till they were approximately 10 cm.

Release-method 1

In order to release and relocate the groupers, this study first experimented with different release techniques. Groupers were marked by fin-clipping their anal fin, but keeping the bony spine intact. Fin-clipping is an easy, cheap, and fast to apply marking technique, which makes it the most suitable marking technique for this research. However, fin regeneration prevents monitoring long-term effects of the restocking project, as released fish whose fin has regrown cannot be identified anymore (Parkos, *et al.*, 2005).

As groupers are territorial animals (Arkive, 2012), it was assumed that they would stay in their habitat once they regard it as their territory. Therefore, four cages (1.6 m x 1.6 m x 1.2 m) were built from fish nets that were placed on the sand bottom in the Abucayan inlet. They were placed at a depth that made sure that they were submerged even during low tide. The nets were adhered to four poles, each in one corner, and each net was approximately 1 metre away from another. The nets were open at the bottom, and the edges were kept into contact with the sea bottom by rocks and weights. A tube with a diameter of 8 cm and a length of 20 cm functioned as an artificial shelter place, and was placed diagonally inside the cage with one end buried in the sand.

Four groupers were fin-clipped and released, one in each cage respectively. To familiarize them with the new water quality, sea water was flown inside the bucket that was used to transport them. This inflow of seawater was repeated after 5 and 10 minutes, and at 15 minutes the first grouper was released inside cage 1. With time intervals of approximately 30 minutes the other groupers were also released. As groupers are benthic species, it is assumed by La Mesa, *et al.* (2008) that it is better to release the individuals close to their new potential habitat. Therefore, they were released inside the cage as close to the sand bottom as possible.

Grouper presence and behaviour was monitored at regular time intervals from outside the cage during daytime. The following day the nets were removed, approximately 24 hours after the groupers were released.

Predator-avoidance experiment

The remaining groupers were transferred from the tanks in different cages inside the bay eight days later. The cages were made by nets with sizes of 4 m x 4 m x 3 m, and their structure was held into position by metal frameworks present in the Abucayan inlet and owned by the Centre for Sustainability. Weights were placed in every corner in order to keep the bottom of the net down and the structure stable. Mesh size of the nets was approximately 1 mm. Two cages were prepared, right next to each other: one for the control setup and the other one for the predator setup. In the predator setup a smaller cage (1m x 1m x 1m) was placed, which was made from plastic and which would later contain a predator. The mesh size allowed the juvenile groupers to enter and leave the cages, but would not allow passage for the predator. The predator cage was placed more or less in the centre of the net, and the top edge was just above the water level. The whole construction was tied to the metal framework.

Grouper juveniles were transported from the hatchery towards the cages in a polystyrene box (80 cm x 30 cm), which was constantly aerated during transportation. They were released with a scooping net. Forty-six individuals were released in the control setup and fifty individuals were released in the predator setup. The difference was to make sure that some individuals from the control setup could be used for experimenting with a new release method, which will be described in 'Release-method 2'.

The following day a predator was released inside the smaller predator cage. Due to availability, a larger, adult brown-marbled grouper (+- 30 cm) was chosen as predator. Brown-marbled groupers are known to be cannibalistic (Hseu, *et al.*, 2007), thus it was assumed that the larger individual would predate upon the smaller ones if they would enter its cage. Also, most grouper individuals already have had optical or physical experience with cannibalism while they were reared in the tanks. Thus, it was assumed that odour cues coming from a larger individual invoked a predator-avoidance response.

Grouper juveniles were daily handfed three times, morning, around noon, and late in the afternoon. They were fed with the same pellets that were used in the hatchery. Foraging behaviour was monitored while being inside the cage when feeding them around noon.

Grouper behaviour was monitored daily for two weeks, with exception for the weekend. This was done by entering the cage with a mask and snorkel twice a day; morning and afternoon. Next, I would refrain myself from making unexpected and large movements by mainly drifting inside the cage motionless. Wave action was mainly blocked by the net, so the impact of the currents while observing was limited. Groupers were observed for more than half an hour, followed by taking pictures of the locations with groupers present. A waterproof Nikon camera was used to make the pictures and in special occasions for filming. Making pictures often ended in coming too close to the grouper juveniles, which would startle them. The observations of their flight response were also monitored.

Release-method 2

A new method was designed to release the groupers in such a way that they could be monitored for the first couple of days after release. The trait that brown-marbled groupers are territorial fish (Arkive, 2012) was used in order to relocate the released groupers with a different method that required less effort to conduct. Groupers were familiarized with plastic tubes with a diameter of 8 cm and 20 cm in length for one week. Ten individuals from the control group were released in two of those tubes and they were placed on the sand bottom near the coral reef. The tubes were placed vertically, and one side was sealed by a fishing net, while the other, open side was placed on the sand bottom. A weight was adhered to the two tubes in order to place them steady on the bottom. After approximately 24 hours the two tubes were placed horizontally and the fish could leave the tubes.

When placing the tubes on the sand bottom the majority of the grouper fingerlings already escaped. The remaining groupers were observed directly after release and that same afternoon. The following day the tubes were monitored in both the morning and afternoon. After two days the tubes were checked for the presence of any grouper individuals again.

This release method was used on a larger scale with all the fish from the predator-avoidance training and from the control setup. In order to make less disturbance while monitoring the groupers, scuba diving gear was used instead of just snorkelling gear. This way observations could be made without making any motions. On the first day the groupers were released by using two constructions, which both consisted of six tubes and two weights (fig. 1). One side of the tubes was again sealed by a net, the other side was temporarily closed by a bamboo lit. In total, twelve groupers from the control setup were left, and they were distributed over the six tubes. Of the predator setup, 28 grouper juveniles were still present and they were also distributed over the six tubes.



Figure 1: Construction used to release the groupers after the predator-avoidance experiments.

During transportation of the constructions from the raft to the release location some groupers escaped. The bamboo lit was supposed to prevent this from happening, however the groupers managed to escape through small gaps between the bamboo and the tubes when the construction bended. Again, after approximately 24 hours the lit was removed and the behaviour of the groupers was observed for two days both in the morning and afternoon. Coral structures were used to conceal our presence from the groupers to some extent after they were released. The second monitoring day was mainly spend in order to relocate the grouper juveniles that were released by scanning the surrounding reef environment.

Habitat surveys

A good understanding of the natural habitat and the population dynamics is essential for a successful stocking program (Bell, *et al.*, 2008). For two weeks the coral reef in the property of the Centre for Sustainability in the Abacuyan inlet was monitored. Every morning and afternoon observations were made by swimming over a large part of the coral reef present in that area. Photos were taken and fish species were determined to species level when possible by using the book: Reef Fish Identification; Tropical Pacific; by Gerald Allen, Roger Steene, Paul Humann, and Ned DeLoach; New World Publications (2003) and Snorkeler's Guide to Marine Life of the Philippines; by Lee Goldman; the National Library of the Philippines (2012). The environment was also scanned for a suitable habitat for the grouper juveniles and possible predators and competitors.

Results

Release-method 1

One grouper was released in every cage with regular time intervals of approximately 25 minutes. Each cage contained gobies (Perciformes, Gobiidae), and cage 2 also contained a white-shouldered whiptail (*Pentapodus bifasciatus*). Cage 1 and 2 were located closer to the coral reef and were further offshore, while cage 3 and 4 contained more seagrass. On 11-10-2012 the four groupers were released and the cages were monitored during that whole day.

11-10-2012

- 9:30 Releasing grouper 1 in cage 1. After release it immediately swam away and hid itself.
- 9:36 No sign of grouper 1.
- 9:49 Grouper 1 was present, but immediately swam away once he spotted me and it hid beneath the net at the edge of the cage.
- 9:56 Releasing grouper 2 in cage 2. After release it immediately swam away and hid itself.
- 10:02 Grouper 2 was resting on the sea bottom and it did not show any reaction towards my presence.
- 10:13 No signs of groupers 1 and 2.
- 10:20 Releasing grouper 3 in cage 3. The grouper first showed freezing behaviour, and after approximately 20 seconds it swam away.
- 10:32 No signs of groupers 1 and 2. Grouper 3 was resting on the sea bottom, but swam away from my presence and hid itself between the seagrass.
- 10:42 Releasing grouper 4 in cage 4. After release it swam into the tube that was placed as a shelter place.
- 11:00 – 11:17 The cages were checked five times with regular time intervals. Only at 11:04 grouper 2 was spotted inside cage 2, but it quickly fled after it was spotted and hid at the edge of the cage.
- 11:27 – 11:46 The cages were checked five times with regular time intervals. No grouper was spotted.
- 14:10 – 14:40 The cages were checked five times with regular time intervals. No grouper was spotted.
- 14:55 – 15:24 The cages were checked five times with regular time intervals. No grouper was spotted.
- 15:40 – 16:12 The cages were checked five times with regular time intervals. No grouper was spotted.

At 11:00 o'clock and during the whole afternoon a great barracuda (*Sphyraena barracuda*) was present above the cages while observations were made.

12-10-2012

- 09:17 – 10:35 The cages were checked five times with regular time intervals. No grouper was spotted.
- 09:36 Cage 1 was removed. No grouper was spotted.
- 10:05 Cage 2 was removed. No grouper was spotted.
- 10:21 Cage 3 was removed. No grouper was spotted.
- 10:45 Cage 4 was removed. No grouper was spotted.
- 11:00 The areas where the cages used to be were checked. No grouper was spotted.

The whole morning the great barracuda was regularly present around the cages while observations were made or when cages were removed.

15-10-2012

- 10:00 The areas where the cages used to be were checked. No grouper was spotted.

Predator-avoidance experiments

23-10-2012

After release in the cage, groupers mainly remained motionless in the water, a behaviour called freezing (La Mesa, *et al.*, 2008). This behaviour was often followed by returning back into the scooping net (fig. 2), which provided shelter and where most of the time other grouper individuals were still present.

After the groupers were released in the cage they typically displayed schooling behaviour (fig. 3). They all avoided the open water, and most of them were lying against the edges or on the bottom of the cage. When they were alone, they often went to other conspecifics as fast as possible. Exploring behaviour was very low, and most groupers either stayed close to the place where they were released, or swam to the deeper parts, especially the darker corners.



Figure 2: Scooping net used to release the grouper fingerlings



Figure 3: Schooling behaviour displayed by the grouper fingerlings shortly after release.

24-10-2012

Groupers were mainly hiding under or behind the weights in the corners, or within folds of the net. Most groupers that were located in the more open areas tried to quickly find shelter in the corners or within folds when they became disturbed.

In the afternoon some groupers displayed a new behaviour: they were gulping at the surface, as if trying to breath air (fig. 4 & fig. 5). Staying at the surface makes the groupers prone to predators, such as kingfishers and herons, which both were observed to use the metal frameworks to hunt for food. When the predator was released, the grouper juveniles did not display any predator-avoidance behaviour.



Figure 4: Gulping behaviour displayed by the grouper fingerlings, predator setup corner 4



Figure 5: Gulping behaviour displayed by the grouper fingerlings, predator setup corner 3

25-10-2012

A difference was observed between the control group and the predator group. In the control group, grouper fingerlings were scattered on the bottom, and were no longer only hiding in corners and

fold. They were also more active and were observed to swim around on the bottom. In the predator setup, groupers still mainly hid in corners and only one was observed in the open area on the bottom. Both cages had one corner where groupers were observed to approach the surface; corner 3 for the control setup and corner 4 for the predator setup (appendix I).

This time I observed the fish while I was inside the cage when I fed them. In the control group, no fish foraging behaviour was observed. In the predator group, very little foraging behaviour was observed. Pellets were released in the corners near the surface. Fish were mainly hiding in the corners at the bottom, so when the pellets sank they would drop right on top of the fish. However, most of them simply ignored the pellets, while some only would eat them when they were sinking right in front of them. None of the grouper juveniles displayed active foraging behaviour that was observed in the hatchery.

26-10-2012

A difference in activity and dispersion was observed between the two setups. Grouper juveniles from the predator setup (fig. 6) were hiding around the weights and were mainly inactive. When they got startled they would frantically try to hide and they would sometimes push others away to hide even better. Their activity remained low for long time periods, which was in contrast with groupers from the control setup. They sought shelter when I entered the cage, but came out relatively soon again (fig. 7). Their activity was also higher as they were swimming around along the bottom, edges and they would sometimes go further into the open water. This particular activity was not observed in the predator setup.

The predator setup had one grouper individual swimming close to the surface. This looked similar to the gulping behaviour described at 24-10 and this grouper showed limited response to my presence. The control setup had multiple individuals approaching the surface, however their behaviours were not different from groupers swimming on the bottom. They would also immediately swim away when I came too close to them and they never stayed at the surface for longer time periods.



Figure 6: Predator setup corner 2

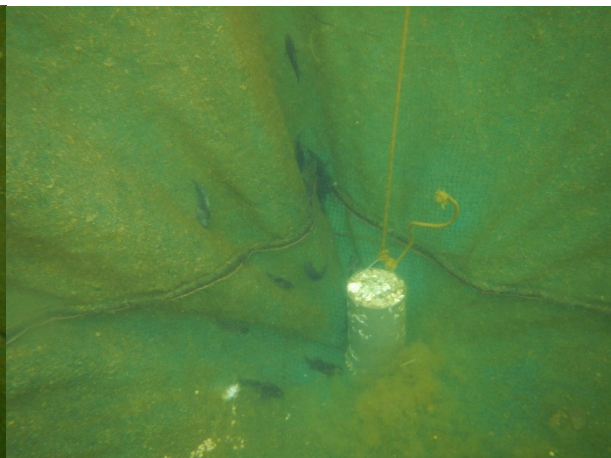


Figure 7: Control setup corner 2

30-10-2012

Both setups contained some corpses of, most likely, brown-marbled grouper. The size of the corpses correspond with the grouper fingerlings, but were in a deteriorated state that did not allow further identification. The control group had three casualties, while the predator group had only one.

Groupers from the predator setup were observed in the corners and some were staying higher in the water column (fig. 8). However, groupers in the control setup were more free swimming and appeared sooner from their hiding places after they received a large disturbance (fig. 9). Few groupers in the predator setup left their shelter, and those that did quickly hid away again when they were approached in order to make pictures. The ones from the control setup also hid when they were approached, but the distance between the observer and the grouper individuals was closer, and they took shelter in a less frantic way.

Both experimental setups had one individual that swam from one corner to another. This is a low amount compared to observations made in the previous week.



Figure 8: Predator setup corner 2



Figure 9: Control setup corner 1

31-10-2012

Within the predator setup clear differences in behaviour were noticed between the different corners. In corner 1 fish were lying quietly on the bottom or weight (fig. 10). In corner 2 fish were aligned in the corner, however there was little activity and most remained in their place unless disturbed (fig. 11). There was also a difference in the colour that was displayed between the two corners: in corner 1 the groupers had a grey colour with dark blotches, while in corner 2 they were completely black.



Figure 10: Predator setup corner 1



Figure 11: Predator setup corner 2

The same applied to the control setup, where groupers in corner 1 were either lying on the bottom around the weight or on the weight itself, while in corner 2 groupers were present higher in the water column than in the other corners. Groupers were also more abundant in corners 1 and 2 compared to corners 3 and 4 in both setups. Although sometimes groupers were present in corners 3

and 4, their numbers rarely exceeded five individuals. This was in contrast to corners 1 and 2, where at least ten individuals were present every time they were monitored. Also, groupers were observed to move from corner 1 to corner 2, and vice versa, regularly. Only in rare occasions did groupers swim to other corners.

1-11-2012

The groupers in the control setup could be found everywhere lying on the net or swimming higher in the water column close to the net. Some were lying completely in open areas resting on the bottom of the net. They could be approached relatively close before swimming away calmly. Even around corners 3 and 4 grouper individuals were observed to swim higher in the water column compared to previous days.

A lot of snappers and scats (*Scatophagidae*) were observed around the cage. Turbidity was low, and the snappers outside the cage were clearly observing the groupers inside the cage. However, the groupers did not seem to be bothered by the wild fish outside the cage.

In the predator setup the groupers were lying against the net in corner 2 all along the water column (fig. 12). Again, this was only observed in corner 2, and in the other corners groupers were resting around or on the weight. Corner 4 harbored more grouper individuals in comparison to other days (fig. 13), but the amount was still low compared to corners 1 and 2.

Groupers in corners 3 and 4 still did not display any foraging behaviour, however in corner 1 and 2 there were some that ate the pellets. Groupers in the control setup were more active in foraging and also swam away from the net in order to get a pellet. In the predator setup groupers would eat a pellet if it would drop next to the net, as they would not swim into the open water.

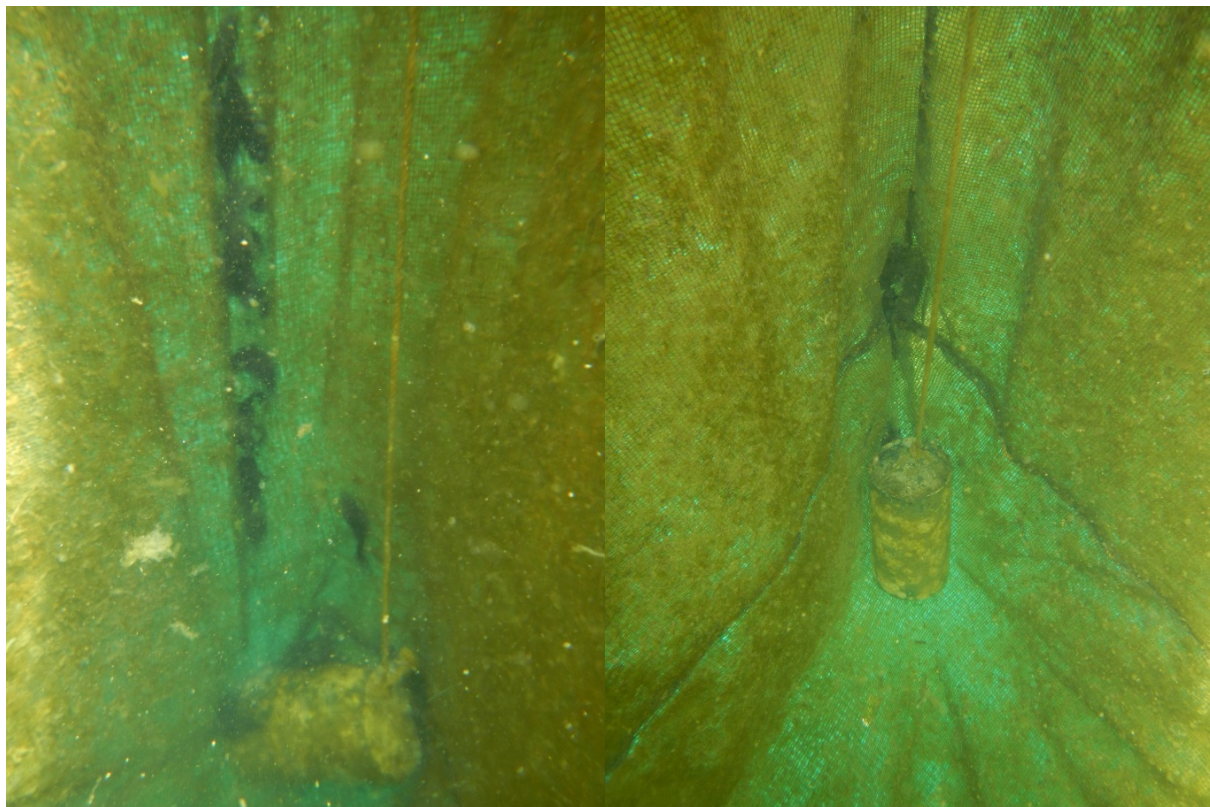


Figure 11: Predator setup corner 2

Figure 12: Predator setup corner 4

2-11-2012

The difference between predator and control setup is becoming less obvious. The proximity before the fish get startled because of me is still higher in the control than in the predator setup. However, fish are becoming more active in the predator setup as well, mainly in corner 2. This is reflected by

the fact that I found some grouper individuals in the tubes in the centre of the cage of the predator experiment as well; 2 in the morning and 3 in the afternoon.

The feeding in corner 2 was successful in both setups, however foraging behaviour is absent in the other corners. This counts for both the control and predator setup. Sunlight is especially aimed at corner 2 in the afternoon, however in the morning when corner 2 is in the shade the groupers are more active as well.

Groupers display more exploring behaviour in the control cage. They explore the open water more often, but also more open bottom areas. While their movements used to be mainly confined to the folds and edges of the net, now they are present almost everywhere. Although the open water is still avoided, larger distances from the sides and corners are made towards the centre. Their numbers inside the tubes were 5 in the morning and 7 in the afternoon.

In the morning I observed a lot of snapper attacks from outside the cage on the groupers inside the cage. Especially in the control group, groupers often got startled because of a snapper attacking them. Snappers also are often hanging around the corners, observing the groupers. This is not the first time I observed snapper attacks, but in my opinion it has become more profound. This might reduce the difference between the control and predator setup, since both are now prone to predator attacks and are used to their new environment.

Release method 2

07-11-2012

In total ten groupers from the control group were equally divided over the two tubes of the construction. Half of the groupers escaped when placing the tubes on the sand bottom near the coral reef. Many of the groupers that escaped swam away, however two individuals remained around the tubes even though they were free. The tubes were sealed by placing the open end on the sand bottom with three and two groupers respectively still inside the two tubes.

In the afternoon two groupers that had already escaped were still present around the tube construction. No other grouper juveniles that had accidentally escaped could be found in the area.

08-11-2012

The tube construction was placed horizontally, which made it possible for the captured groupers to get out of the tubes. Two groupers that had accidentally escaped the previous day were present on top and next to tube construction. After release, the grouper individuals quickly swam out of the tubes and started to explore the environment.

09-11-2012

Four groupers were still present inside or under the tubes. On top of a small coral reef structure a third grouper juvenile was lying. This grouper juvenile hid inside the coral reef structure when it was startled, and came back out again after some time. A fourth grouper juvenile was resting on the sand bottom a couple of metres away. A staghorn damselfish (*Amblyglyphidodon curacao*) was trying to scare it away by creating small clouds of dust with its tail. When the juvenile grouper swam away it went to another small open sandy area where it rested on the bottom. After some time observing it, I tried to scare it towards the tubes, but it deliberately avoided that area and it swam off further away.

14-11-2012

Twelve groupers from the control setup and 28 from the predator setup were left after the experiments and they were released in the tube constructions. The tube constructions were again not sealed well, and a lot of grouper individuals escaped preliminary. Approximately three groupers and 24 of the control and predator setup, respectively, were still in the tubes when they were placed on the sand bottom. The high escape rate of the control setup groupers was because the heavy weight of the tube construction made transportation unstable. During the transportation of the

predator setup groupers the tube construction was held in a different position, which made it more stable.

15-11-2012 Morning

After removing the bamboo wood used to seal the tubes, the groupers and their direct environment were monitored for approximately one and a half hour. Grouper juveniles from the control setup did not show much activity, and mainly hid completely in the back of the tubes. After approximately half an hour the tube construction of the control setup was carefully checked again. Only two grouper individuals were still present, hiding in the back of the tubes. After approximately half an hour they were checked again, and only one individual was left, hiding in the back of a tube.

For the predator setup, four different phases in grouper familiarization behaviour with their new environment could be distinguished.

First phase: Groupers were mainly hiding inside their tubes and showed little activity.

Second phase: Groupers started to actively move from one tube to another. Most of them were quickly switching tubes, minimizing the time spend on swimming in the open water. They still avoided swimming away from the tubes and they would only shift to another tube that was next to theirs.

Third phase: Groupers were actively exploring the environment of the tubes. They no longer stayed in the tubes as much as possible and sometimes lay in front, on top, or aside from the tubes. Other wild fish started to come closer to the tubes and showed interactions with the groupers. When groupers went too close to the corals they were often scared away by wild fish. Schooling behaviour was also typical for this phase: when one grouper swam away or showed activity, it invoked higher activity among other individuals until they all had calmed down and would rest in or around the tubes again.

Fourth phase: Groupers actively started to explore more of the environment. They were no longer bound to stay around the tubes, but would still return to them when startled. They clearly noticed our presence, but started to become less bothered by it. Wild fish were less interested in the released groupers compared to the previous phase.

15-11-2012 Afternoon

Groupers were mainly resting on the bottom of the tubes or under the tubes. Two grouper individuals were present under the tubes of the control setup. This place was not checked in the morning, which means more might have been present in the morning. These two groupers did not show any activity.

Groupers from the predator setup showed very little activity, and most activity was displayed when my diving buddy came to close for making observations or when their numbers were counted. It was found that approximately 12 to 15 grouper individuals were still present in the predator setup. Their behaviour when my diving buddy came to close was swimming quick, but short distances. Sometimes from one tube to another and sometimes more towards open areas. When they were startled while on sandy bottom, they often left a cloud of dust behind. This was not observed when they were swimming to another location when they were not startled. No exploring behaviour was observed.

16-11-2012 Morning

Only one grouper individual was present in a tube of the control setup. This grouper individual did not display any activity and remained in its tube while being monitored. One other grouper was found resting on a piece of coral a few meters away.

16-11-2012 Afternoon

We spend approximately half an hour to relocate the groupers. The one from the control setup was still present, this time hiding under the tubes. We could not find any other grouper individual.

Habitat surveys

The species that were spotted in the coral reef where the grouper juveniles were released and which were seen more than twice are listed below. This list is far from complete, as a lot of species were keeping a large distance from the observer or could not be determined from photos taken. Still, the most common species that were observed a lot are included in the list.

Disk & Large Oval Fishes

<i>Chaetodon lunulatus</i>	Redfin butterflyfish
<i>Chaetodon octofasciatus</i>	Eight-banded butterflyfish
<i>Forcipiger flavissimus</i>	Longnose butterflyfish
<i>Chaetodon vagabundus</i>	Vagabond butterflyfish
<i>Chelmon rostratus</i>	Long-beaked coralfish
<i>Heniochus acuminatus</i>	Longfin bannerfish
<i>Chaetodontoplus mesoleucus</i>	Vermiculated angelfish
<i>Chaetodon oxycephalus</i>	Spot-nape butterflyfish
<i>Acanthurus auranticavus</i>	Orange-socket surgeonfish
<i>Saginus guttatus</i>	Golden rabbitfish
<i>Saginus virgatus</i>	Barred rabbitfish
<i>Saginus vulpinus</i>	Foxface rabbitfish

Small Oval Fishes

<i>Amphiprion ocellaris</i>	False clown anemonefish
<i>Dascyllus aruanus</i>	Humbug dascyllus
Pomacentridae spp.	Damselfishes
<i>Amblyglyphidodon curacao</i>	Staghorn damsel
<i>Dischistodus perspicillatus</i>	White damsel

Sloping Heads/Tapered Bodies

<i>Diagramma melanacrum</i>	Indonesian sweetlips
<i>Lutjanus fulviflamma</i>	Longspot snapper
<i>Lutjanus monostigma</i>	Onespot snapper
<i>Lutjanus argentimaculatus</i>	Red snapper
<i>Lutjanus decussatus</i>	Checkered snapper
<i>Pentapodus bifasciatus</i>	White-shouldered whiptail
<i>Pentapodus trivittatus</i>	Three-stripe whiptail
<i>Scolopsis ciliata</i>	Whitestreak monocle bream
<i>Monotaxis grandoculis</i>	Humpback big-eye bream
<i>Caesio teres</i>	Blue and yellow fusilier

Silvery

<i>Platax teira</i>	Longfin spadefish
<i>Sphyræna qenie</i>	Blackfin barracuda
<i>Sphyræna barracuda</i>	Great barracuda
<i>Scatophagus argus</i>	Spotted scat
<i>Aeoliscus strigatus</i>	Razorfish

Heavy bodies

<i>Cephalopholis boenak</i>	Chocolate grouper
<i>Epinephelus merra</i>	Honeycomb grouper
<i>Epinephelus ongus</i>	Speckled fin grouper
<i>Cephalopholis argus</i>	Peacock grouper
<i>Plectropomus maculatus</i>	Spotted coral grouper

Parrotfishes and wrasses

Scaridae spp.	Parrotfishes
<i>Cheilinus fasciatus</i>	Redbreasted wrasse
<i>Labroides dimidiatus</i>	Bluestreak cleaner wrasse
<i>Halichoeres podostigma</i>	Axil spot wrasse

Bottom-dwellers

Gobidae spp.	Gobies
<i>Koumansetta hectori</i>	Yellowstripe goby
<i>Pterois volitans</i>	Common lionfish
<i>Pterois antennata</i>	Spotfin lionfish
<i>Dendrochirus zebra</i>	Zebra lionfish
<i>Opistognathus dendriticus</i>	Dendritic jawfish
Odd-shaped swimmers	
Ostraciidae spp.	Boxfishes
<i>Rhinecanthus verrucosus</i>	Blackpatch triggerfish
<i>Pseudobalistoides flavimarginatus</i>	Yellow margin triggerfish
Mullidae spp.	Goatfishes
<i>Parupeneus barberinus</i>	Dash-dot goatfish
<i>Pseudomonacanthus macrurus</i>	Strapweed filefish
<i>Lactoria cornuta</i>	Longhorn cowfish
<i>Diodon hystrix</i>	Porcupinefish
<i>Arothron mappa</i>	Map puffer
<i>Arothron manilensis</i>	Striped puffer
<i>Canthigaster papua</i>	Papuan toby
<i>Plotosus lineatus</i>	Striped eel catfish
Eels	
<i>Echidna nebulosa</i>	Snowflake moray

Conclusions

The reared juvenile groupers were able to find locations useful for their settlement relatively quickly. Most groupers already dispersed after one day, and it is unlikely they are all eaten as they could shelter in the tubes. Furthermore, two grouper individuals, which were released in the first attempt with release-method 2, clearly used coral structure as their niche and would hide in it when startled. Also when the groupers were released in the two cages for the predator experiments they would quickly recover from stress and swim to areas which were similar to environments they might use for settlement. Except for schooling behaviour, only a few groupers would display the unnatural 'gulping' behaviour inside the cage.

Still, introducing artificial shelter that can be used by the released groupers is assumed to increase their chances of survival during the first few days. According to Vilhunen (2004) it will take approximately three days for fish to familiarize themselves with a new environment. This is supported by the results of the cage experiment, since it took approximately three days for the groupers of the control group to display behaviour they would more or less maintain during the rest of the experiment. Since the groupers were introduced in their new environment in tubes, they could use this as shelter if needed. After all, no other species inhabited it or was familiar with the new construction that was introduced. Many observations were made of fish, both herbivory and predatory, scaring the released groupers away from specific areas. Besides, most grouper juveniles that accidentally escaped displayed freezing behaviour while still being high in the water column and would not autonomously seek shelter in nearby reef structures (La Mesa, *et al.*, 2008).

Training grouper juveniles to avoid predators can lead to habituation of the predator stimuli and is most likely ineffective. During the first week of making observations there were clear differences in behaviour between the groupers in the predator setup and the control setup. However, these differences quickly diminished and at the end of the second week of making observations groupers of both setups were behaving very similar. According to literature, training for predator-avoidance enhances the survival rate of released fish reared in aquaculture, but this same result can be achieved by repeatedly chasing juveniles (Vilhunen, 2006).

The survival rate was relatively low inside the cages used for the predator experiments. Normally, the survival rate of grouper juveniles released in these cages is approximately 95%. In approximately three weeks time 28 out of 50 groupers (survival rate of 56%) were still left inside the predator setup. In the control setup it was even lower, as 22 out of 64 groupers (survival rate of 34%) in total were released. The difference in survivability between the two setups was supposed to be due to the presence of the predator, and the possibility that groupers from the predator setup could get eaten. However, the survivability of the groupers inside the predator setup is higher compared to the control setup and therefore no firm conclusion can be based on this result.

The groupers displayed impaired foraging behaviour compared to when they were reared inside the tank. Even though they received the same pellets, they would barely make any movements to get some. In the tank, they would all come to the surface in order to be the first one to get a food pellet and they were extremely active to get food. It is known that once reared groupers shift their diet from pellets to live food they cannot shift back to pellets anymore. Inside the cage there was plenty of shrimps, crabs and small fish, organisms that can safely be assumed to serve as prey items. However, whether the reared groupers indeed shifted to predate upon these organisms was not observed and can therefore not be concluded.

Due to the accidental escapement of groupers from the control setup a different comparison could be made. Approximately three groupers from the control setup of the second attempt (released on 14-11-2012) were released in six tubes. This was in contrast to the 5 groupers in two tubes from the first attempt of release-method 2 (released on 7-11-2012) and 24 groupers of the predator setup (released on 14-11-2012). This means the groupers had to share a tube with two to three conspecifics on average. Indeed, a clear difference in behaviour was observed, as the groupers from released in higher densities were far more bold and exploring. They were schooling when they got startled, while the groupers from the control setup in the second attempt could not resort to such

behaviour. This might explain why the groupers that came out fairly quick were far more active, while the groupers from the control setup mainly hid under the tubes.

Food resources are abundant in the area that is property of the Centre for Sustainability. Especially shrimps and gobies are profound and their burrows are visible everywhere in the sandy areas. Prey species for adult brown-marbled groupers, such as goatfishes, herring, and squirrelfish (Binohlan, 2012) are also plenty available.

There are a number of competitor species present in the coral reef ecosystem, but the most abundant ones are likely to be chocolate groupers and common lionfishes. Although the chocolate grouper will not grow as big, they have a similar niche with smaller brown-marbled groupers (Capuli, 2012). Common lionfishes are a widespread infestation, can dominate ecosystems, reduce their prey species by large amounts, and outcompete groupers and snappers (Morris, 2012).

Discussion

Although no firm conclusions could be about the experiment, this report provides some basic knowledge about the ecosystem in the Abacuyan inlet and its interaction with recently released groupers. Since most groupers from the control setup were exposed to snapper attacks and escaped before they were supposed to be released, no comparison about the effect of predator-avoidance training could be made between the two setups. Still, the main objective of this research was to get more insight in the best way to restock brown-marbled groupers in Puerto Princesa bay. Compared to the groupers in the control setup, the predator setup showed a different behaviour inside the cage that might enhance survival in the wild. They were faster in their hiding response, and were more concerned about getting the best hiding spot available when they were startled by the observer.

However, this difference in behaviour was especially profound in the first week after they were released in the setups. This indicates that predator-avoidance behaviour is an innate trait rather than a behaviour obtained by social learning (Brown & Laland, 2001). After the stressful period of release was over, groupers from the predator setup avoided the open, unsheltered areas within the setup. They mainly hid behind and around the weights in the corner, which was in clear contrast to the individuals from the control setup.

After the first week, the difference between the control and predator setup diminished over time. The grouper juveniles from the predator setup became more bold in the second observation week, which indicates that there was some form of habituation. This is in contrast to the findings of Vilhunen (2006), who found an enhanced predator cue-avoidance in arctic charr (*Salvelinus alpinus*) with a significant difference between the first exposure and the fourth. However, the arctic charr were reintroduced to the flow-through fluvium each time, and were kept in isolation trays between the exposures. The grouper juveniles from this experiment were exposed to predator cues day and night for two weeks. Habituation to the presence of the predator in this research would therefore be much more likely compared to the four exposures of predator cues in the study of Vilhunen, 2006. Therefore, it is assumed that prolonged exposure to predator cues negatively affect the predator-avoidance behaviour due to habituation.

The high mortality within the cages indicated that something was amiss in both setups. Especially since the control setup was supposed to have a similar mortality rate as groupers that were reared in the cage by the Centre for Sustainability. Although no firm conclusions can be made about the survival rates, it remains peculiar that the survivability in the control setup (34%) is much lower than the survival rate of the predator setup (56%). After all, the predator was supposed to account for the increased mortality in this setup if that would have been the result. The most probable reason for the high difference is that there was a hole present in the control setup, and groupers could escape from the cage. There is a high chance that they did not survive their escape, there were a lot of predatory species present, e.g. barracudas and snappers. Besides, according to La Mesa, *et al.* (2008) groupers are unable to find suitable areas for settlement on their own when they are not released close to the coral reef structures. The water depth was approximately 16 metres over there, making the chance of survival for these possibly escaped grouper juveniles very unlikely.

It was concluded that releasing groupers in tubes in higher densities could invoke a stronger exploring behaviour and could be the main agent for them to be more bold towards other fish. However, to conclude that releasing higher densities of groupers in the area is for the better would be too rash. After all, brown-marbled groupers are territorial animals that will only form aggregations during spawning season (Arkive, 2012). This was observed in the first attempt of release-method 2 of the control group. Grouper individuals started to distribute themselves over the area after already one day. This indicated that schooling behaviour is used as a protective strategy when stressed, but that they turn to territorial animals when they've become familiar with the environment.

This might also have been the reason that in the second release attempt all groupers from the predator setup left after already one day, while the control setup still housed one individual. Groupers might have linked the tubes with conspecifics, and thus deemed this area as unsuitable for settlement. However, this explanation does not exactly correspond with the observation that there were still groupers present in the tubes after one day of the first attempt of release-method 2. This

might be because there was a large difference in abundance between the first attempt of release-method 2 (7 groupers in total) and groupers from the predator setup in the second attempt (24 groupers). But, this difference could also have been the result of an effect of predator-avoidance training, which made the groupers from the predator setup experienced in living exposed to predator cues. This is not very likely, since both setups had experiences with living in an area exposed to predator cues. Therefore, more research is needed to study the cause for the fast dispersal rate of the groupers from the predator setup.

Recommendations

The coral reef ecosystem in the Abacuyan inlet is most likely a delicate one. There are not a lot of living corals anymore and depths lower than ten metres are dominated by mud wastelands. However, there is still a considerable level of fish biodiversity in the coral reef, eventually supported by the corals. This report already provided some basic assessment of the ecosystem, but a quantification of the organisms is essential to visualize the stock dynamics and the possible impacts of the restocking programme.

During the observations in the area no wild brown-marbled groupers were spotted, and it is safe to assume that the reintroduction of a species with a trophic level of 4.1 (Binohlan, 2012), which is considerably high, can restructure the foodweb. Therefore, a monitoring programme of the coral reef ecosystem is essential in order to: 1) perceive adverse changes rapidly, and 2) take appropriate measures quickly. After all, the restocking is meant for restoration of the ecosystem, not to invoke a drastic decline in biodiversity.

Carrying capacity should be kept in mind when restocking a specific amount of hatchery-bred groupers. Groupers are territorial species and the restricted resources of the habitat result in a limited carrying capacity (La Mesa, *et al.*, 2008). Therefore, it is recommended to link the amount of groupers released at a time on this carrying capacity. If not, released conspecifics might start to hamper each other's survival rate, which is not in the best interest of the restocking programme.

The hatchery-bred grouper juveniles showed the innate ability to recognize and adjust accordingly to a predator in the experiment. Training them in avoiding predators for two weeks is therefore expected to be a cost inefficient method to enhance their survival after release. It is assumed that it would be better to focus on conditioning groupers in dealing with stressful situations, since fish that are reared with experiences in environmental variability are more flexible in learning behavioural skills (Braithwaite & Salvanes, 2005).

For restocking purposes it is also interesting to study the effectiveness of hatchery-bred groupers in capturing live prey. What caused the high mortality in the cages is unknown, but since it was observed that grouper juveniles barely displayed foraging behaviour it is suggested that it is starvation. Still, lots of shrimps and small fish were present in the cage, indicating that the groupers lacked the ability to catch a sufficient amount of prey to survive. Based on these assumptions it is safe to say that being unable to catch live prey is most likely the main reason for the high mortality post release. A study is required to support these assumptions.

To create a self-replenishing population of brown-marbled grouper, it is essential to also restore the environment in such a way that they can complete their life-cycle and contribute to spawning biomass. This means their spawning ground has to be a protected area, where the groupers can aggregate without being disturbed. Juveniles should have the availability over a seagrass habitat that provides them with shelter and food resources. There is a small area between the shore and coral reef covered with seagrass, possibly too small to serve as a suitable habitat for grouper fingerlings. This has to be considered when the stocking programme is conducted.

Research should be conducted to find the most effective method of releasing groupers. The observed difference in grouper behaviour after release was most likely due to the different amounts of groupers released in the tube constructions. Grouper juveniles might benefit from being released in higher abundances, since they can resort to defensive strategies such as schooling behaviour. However, it might also put an additional constraint by increasing the competition between conspecifics. Finding the most beneficial release method can possibly enhance the efficiency of the stocking programme. The groupers from the first attempt in release-method 2 (released on 7-11-2012) were considered to be released in the most successful density (7 groupers with two tubes).

Finally, it is recommended to establish some quantitative objectives for the stocking programme (Bell, *et al.*, 2008). This will make the assessment of its successfulness straightforward and it will make interpreting the results easier. Formulating objectives will also aid the analysis of the monitoring, since it makes it possible to evaluate whether releasing hatchery-bred groupers is the right course of action to restore the ecosystem.

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