



3-2015

Further investigation into critical habitat for juvenile dhufish (*Glaucosoma hebraicum*), artificial habitats and the potential to monitor annual juvenile recruitment

Paul Lewis

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**Further investigation into critical
habitat for juvenile dhufish
(*Glaucosoma hebraicum*), artificial
habitats and the potential to
monitor annual juvenile recruitment**

**NRM Project 09038 – Protecting
inshore and demersal finfish.**

P. Lewis



Government of Western Australia
Department of Fisheries

This project is supported by funding from the Western
Australian Government's State NRM Program

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Correct citation:

Lewis, P, 2015. Further investigation into critical habitat for juvenile dhufish (*Glaucosoma hebraicum*), artificial habitats and the potential to monitor annual juvenile recruitment. NRM Project 09038 – Protecting inshore and demersal finfish. Fisheries Research Report No. 265. Department of Fisheries, Western Australia. 80pp.

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Executive Summary

The West Australian dhufish is a demersal fish species that is highly sought after by commercial and recreational fishers in the West Coast Bioregion of WA. An assessment of the stock status in 2009 by the Department of Fisheries indicated the stock was overexploited with a need for a 50% reduction in the overall catch. As the species is long lived and slow growing, reaching a maximum age of 40 years of age, the recovery is likely to be slow. The current size limit restrictions mean fish do not enter the fishery until they are 6-7 years of age although they become vulnerable to capture and hence fishing mortality from around 3-4 years of age. Previous assessments of the age structure and catch data for dhufish demonstrated that towards the southern extent of its distribution, annual recruitment is highly variable, which has implications for managing this species in those areas. Due mainly to data limitations, methods have not yet been applied in stock assessments for this species that involve forecasting of future stock levels (e.g. a statistical catch-at-age model), although use of such methods is planned for future assessments. A key goal towards assessments for dhufish is thus to ensure that sufficient data become available to provide estimates of future population biomass. If a reliable measure of annual recruitment of juvenile West Australian dhufish could be developed, and if it could be shown that this is strongly-related to observed trends in future year class strength in adults, as estimated from annual age composition samples, this would be very valuable for informing future stock assessments for this species.

The current Natural Resource Management (NRM) funded project on protecting inshore and demersal finfish (Project 09038) was initiated in 2010 to gain further knowledge on the critical nursery habitats for dhufish. Knowledge of the nursery habitat types is important for the informed management of the species and is required for the potential monitoring of annual juvenile recruitment. The initial field surveys in 2011 collected a few juvenile dhufish and identified the critical habitat types of marginal sand inundated reef and patchy seagrass beds in locations of dhufish nursery areas. The initial surveys and anecdotal reports identified alternative methods and sites, including artificial habitats that could potentially be used to monitor annual juvenile dhufish recruitment strength. As the initial fieldwork was successful in meeting its objectives the project was given a one year extension to follow up on the initial fieldwork and anecdotal reports, which are covered in this report.

The objectives for the additional year of field sampling in 2012 were to;

1. Regularly monitor, monthly if possible, the abundance, size and behaviour of juvenile dhufish at an abalone lease site off Augusta using stereo diver operated video (sDOV) surveys.
2. Trial the use of small artificial habitats as sites for monitoring dhufish recruitment in the Perth metropolitan area.
3. Assess annual variation in juvenile dhufish abundance by utilising various methods to resurvey the Perth metropolitan trawl area for juvenile dhufish and compare to results from 2011.
4. Investigate additional sites, including established artificial habitats, identified in southwestern WA as potential nursery areas for juvenile dhufish.
5. Participate in a trial for the use of the high throughput sequencing (HTS) technique in WA to investigate the diet of juvenile dhufish and compare with the traditional microscopy identification of stomach contents.

The project successfully completed all of these objectives and although additional sites in southwestern WA were investigated (Objective 4), juvenile dhufish were not observed at any of these in 2012.

The monitoring at the Augusta abalone lease site documented a high abundance of juvenile dhufish (peak abundance = 147 juveniles) and, importantly, determined that sDOV transects were a suitable method for monitoring juvenile dhufish abundance. The monthly sDOV monitoring surveys indicated the peak in abundance of juvenile dhufish at the site occurred in the November-February period, when juveniles are approximately 12 months of age. The stereo video technique proved highly effective for juvenile dhufish and allowed the size of most juveniles to be estimated accurately and hence growth of the juveniles at the site could be tracked over the year and annual cohorts discerned. The surveys indicated juveniles utilised the lease site from less than one year of age and 80 mm in length until they were approximately two years of age and 240 mm in length. The occurrence of juvenile dhufish at this low profile hard substrate in predominantly sand areas and subsequent shift away from this habitat as they reach approximately two years of age correlates with previous studies showing dhufish shift habitats as they grow.

The study documented schools of juvenile dhufish numbering over 120 individuals from the size of 150 mm in length. It also showed the “preference” of the juvenile dhufish school for occurring in the vicinity of the 30 cement pipes laid out in a V formation or “V-pipes” section of the lease site. This information can be utilised to design an annual recruitment monitoring survey and potential design for future placement of artificial recruitment monitoring sites (ARMS) at additional sites along the WA coast. The monthly surveys clearly showed the discrimination of annual cohorts in the length frequency data and possible early and late season spawned cohorts within each year class. The video surveys also documented important behavioural information on juvenile dhufish including information on the solitary and agonistic behaviour among smaller juveniles, and further confirmation that their critical nursery habitat includes *Zostera* sp. eelgrass and *Posidonia* sp. seagrass beds.

The high abundance of juvenile dhufish at the Augusta artificial habitat site may coincide with a high annual recruitment to the area due to favourable environmental conditions with the recruits attracted to the new artificial habitat and the refuge plus food resources it provides. The high numbers at this site may also indicate there is a shortage of low profile hard substrate in predominantly sandy habitats within this area. This may be a limiting factor in annual dhufish recruitment to some areas and the addition substantial ARMS may enhance the localised dhufish recruitment. A long term study establishing substantial recruitment monitoring sites along the coast may also have the added benefit of enhancing localised recruitment which could be assessed through a Before After Control Impacted (BACI) designed study monitoring dhufish abundance on surrounding natural reefs areas in the vicinity of ARMS and control sites.

The trial of the use of small artificial habitats (masonry bricks) in the Perth metropolitan area was successful in attracting juvenile dhufish. In comparison to the current and previous larger scale surveys involving multiple methods, of trawl, fish trap, towed video and BRUVs in the nearby vicinity was much more effective with a similar return of four juvenile dhufish for considerably less effort required. Thus, the ongoing use of artificial habitats to monitor juvenile dhufish abundance in the area is recommended over the continuation of larger scale surveys. The large scale survey for juvenile dhufish conducted during the project extension, in 2012, of the Perth metropolitan area scallop trawl area, found similar low numbers of juvenile (1 year old) dhufish to the previous 2011 and 2003 surveys. Thus, it appeared that the abundance and hence recruitment of the 2011 spawned year class of juvenile dhufish in the Perth metropolitan area during 2012 was at a similar low level to the 2010 year class, at least in areas near the survey sites. It is proposed that this cohort be followed through in the future to compare to the relative abundance of the adults in that year classes, as determined by age structure monitoring.

The search for additional juvenile dhufish nursery areas in the Geographe Bay region from anecdotal reports by fishers and divers was not successful and time consuming. The sites investigated were generally reported as locations where juvenile dhufish had been observed, were suitable sand inundated marginal reef habitat or established artificial habitats that could be used for ongoing monitoring. The sites were surveyed by multiple methods and the benthic habitat types and fish species present at each of these sites are described with a number of the sites suitable for juvenile dhufish. Further surveys in years of high juvenile dhufish recruitment or the deployment of ARMS in these areas may reveal the potential of these sites for monitoring annual juvenile dhufish recruitment in the Geographe Bay area.

The project also included an additional study of the diet of juvenile dhufish, investigating the potential use of data on prey items to infer on habitat utilisation by dhufish. The identification of prey items was carried using the traditional prey hard part visual identification technique in parallel with molecular High Throughput Sequencing (HTS) techniques. Results showed that juvenile dhufish consumed a wide range of at least 20 different prey items associated with sandy, pelagic and seagrass habitats. The comparison of traditional and molecular techniques indicated the potential of HTS for such studies; however the technique is currently limited by the shortage of sequences of WA species in the existing databases.

In summary, the success of the Augusta abalone lease monitoring and the small artificial habitats established in the Perth metropolitan area for attracting juvenile dhufish provide encouragement for the potential of specific artificial habitats or ARMS to be used to monitor annual juvenile dhufish recruitment or potentially enhance localised recruitment. The site assessment, and establishment of ARMS in the “V-pipe” design at a number of locations in a range of depths along the WA coast, with regular diver sDOV or ROV surveys to coincide with the period of peak abundance from November to February each year, has the potential to provide a spatially robust annual juvenile recruitment index for WA dhufish. The initial costs of establishing similar sized sites to the V-pipe configurations found off Augusta would be substantial in vessel and diving personnel time. However, if the ongoing monitoring of dhufish recruitment provided by such an established network of monitoring sites is reflected in the adult age structure it would be valuable for the monitoring, assessment and sustainable management of the species.

Ideally ARMS would be established in regions such as the Houtman Abrolhos Islands, Perth metropolitan, Geographe Bay and possibly off Lancelin along with the Augusta site to monitor the annual spatial variability in juvenile dhufish recruitment. Monitoring nearby natural recruitment sites and surrounding natural reef sites by regular sDOV surveys would have the added benefit of assessing potential localised recruitment enhancement to the area by the ARMS. Such regular monitoring surveys could potentially yield recruitment information, range extensions or detections of other important species. The current study also yielded information on the growth and recruitment of other species, and on the range expansion of species such as the tropical serranid, Rankin cod (*Epinephilus multinotatus*) recorded at the Augusta site. The development of such a study into dhufish recruitment index and recruitment enhancement requires a long term commitment as it is likely to take at least 10-15 years to establish whether the juvenile recruitment index can provide a good prediction of future year class strength and any localised recruitment enhancement is observed, in part because dhufish do not become fully recruited into the fishery until they are about 7-10 years old. Nevertheless, the benefits for management of the stock would be increased knowledge of dhufish biology and potentially improved stock assessments.

1.0 Background

This report is the third in a series produced under a Natural Resource Management (NRM) funded project, which commenced in 2009 to identify the nursery areas of West Australian dhufish (*Glaucosoma hebraicum*). The project was initiated because limited information was available on the nursery habitats of juvenile dhufish and their distribution. Dhufish are an iconic commercial and recreational species in the West Coast Bioregion (WCB) and are used as a key indicator species for the demersal finfish suite. Knowledge of their nursery areas was deemed critical for the ongoing monitoring of the species. However, given the low number of previously recorded juvenile dhufish (TL<150 mm), this project was regarded as high risk.

The project involved an initial workshop, which collated available scientific and anecdotal information at the time on the biology and ecology of the WA dhufish, including juvenile recruitment areas, and recommended a field plan that utilised a wide variety of sampling techniques to identify the critical nursery habitat for the species (Mitsopoulos and Molony 2010). The subsequent field program, in 2010-11, was limited mainly to the scallop trawl area in the Perth metropolitan region, where juveniles had been encountered previously (Hesp *et al.*, 2002). The sampling was successful in collecting a few juveniles, identified the nursery habitat on which they were occurring as marginal sand inundated reef and *Posidonia* sp. seagrass beds and identified sampling techniques for the juveniles other than trawling (Lewis *et al.* 2012). The report also collated anecdotal information and examined Research Angler program logbook data on captures of undersize dhufish to identify a number of additional sites requiring further investigation that were deemed potential dhufish nursery areas. It provided recommendations on follow up fieldwork including a repeat of the sampling in the Perth metropolitan area to determine the degree of annual fluctuations, the regular surveying of juveniles located at an abalone lease off Augusta, a trial of the effectiveness of artificial habitats in the Perth metropolitan area and the investigation of further areas outside the Perth metropolitan region for the occurrence of juvenile dhufish.

The project then followed up on these recommendations through the provision of additional top-up funding provided by NRM in 2011. By applying these recommendations, the project extension was able to gather further critical information on juvenile dhufish with particular emphasis on researching the abundance of juveniles reported at the artificial abalone habitat off Augusta. The overall goal of the extension was to assess the feasibility of developing a method for monitoring annual juvenile recruitment of dhufish.

1.1 Dhufish biology

Dhufish are endemic to the lower west coast of WA from Shark Bay to Recherche Archipelago (Hutchins and Swainston 1986). They are a large (maximum size and weight of 1200 mm TL and 26 kg) and relatively long-lived species (maximum age recorded 41 years), but matures at a relatively young age (3-4 years) and small size 300-350 mm in TL (Hesp *et al.* 2002).

Dhufish are a multiple batch spawner that mainly generally spawn from December until March, although spawning can commence as early November and continue until April, i.e. ~ a period of 6 months (Hesp *et al.* 2002). Larval duration is approximately 45-46 days although it has been noted that the eyes become sensitive to light and adapted to nocturnal feeding when larvae are ~ 3 weeks old, at which time, they become light sensitive and descend in the water column to the seabed (Pironet and Neira 1998, Shand 2001). The currents of the west coast of WA at the time of dhufish spawning can flow both northwards inshore (Capes current) and southwards

offshore (Leeuwin current) (Lenanton 2009), thereby presumably aiding their dispersal of eggs and larvae. Hydrodynamic modelling suggests an additional inshore movement of the bottom waters where dhufish larvae are likely to reside, enabling their retention and settlement in adjacent coastal waters (Strezlecki *et al.* 2012). The recent sampling of dhufish eggs and larvae in waters of the Capes and Perth metropolitan regions indicate they are widespread through inshore coastal waters to depths of 50 m (Strezlecki *et al.* 2012). DNA studies indicate a single genetic stock exists through the WCB. Although otolith microchemistry suggests limited movements of adults along the WCB (Fairclough *et al.* 2012), recent genetic and hydrodynamic modelling studies indicate that dhufish larvae disperse widely (Berry *et al.* 2012).

The protracted spawning period, relatively long larval stage and strong currents in both northward and southward directions, depending upon location across the shelf, can theoretically result in a cohort of dhufish recruiting to a particular area ranging in age by up to six months, and potentially from a range of areas along the coast. Evidence for this can be seen in the 1 year old juveniles recorded by Hesp *et al.* (2002) in the Perth metropolitan area which ranged in size by up to 70 mm each month.

Previous studies on the species have indicated that, initially, small juvenile dhufish occur in sandy areas over hard substrate near reefs (Hesp *et al.* 2002, Platell *et al.* 2010). At around 150 mm TL, dhufish were thought to move to low relief reef habitats and then, at the approach of maturity, ~ TL 300 mm, fish move to higher relief reef habitats. The move of dhufish from their initial habitat was also associated with a change in diet, from predominantly of small invertebrates to mainly fish (Hesp *et al.* 2002, Platell *et al.* 2010). As in the former study, the initial field program of this project identified the occurrence of juvenile dhufish (TL<150 mm) on sand inundated reef, but in addition, juvenile dhufish were found in patchy deepwater seagrass beds off the Perth metropolitan area. The survey mapped these habitat types in the Perth metropolitan trawl area and identified large areas of such habitat types (Lewis *et al.* 2012).

Further details on the biology of dhufish have been summarised, in the previous reports by Mitsopoulos and Molony (2010), Lewis *et al.* (2012) and Smallwood *et al.* (2013).

1.2 West Coast Demersal Scalefish Resource

The West Coast Demersal Scalefish Resource (WCDSR) includes approximately 100 demersal scalefish species in the WCB taken by both recreational and commercial fishers. Dhufish attain a reasonably large size and are highly regarded for their eating qualities and so are targeted by both fisheries. Dhufish is one of the most important species and is one of three indicator species for the demersal “suite” of species, along with snapper (*Pagrus auratus*, now *Chrysophrys auratus*) and baldchin groper (*Choereodon rubescens*). The annual catch for the species in 2011 was approximately 73 t, 13 t and 74 t for the commercial, charter and recreational sectors, respectively (Fairclough *et al.* 2013).

The age structure of the species is monitored annually and is currently assessed on the basis of estimates of fishing mortality and related measures from per recruit analyses, together with trends in catches (i.e. employing level 3 assessments). The assessments in 2007 and 2009 indicated that overfishing was occurring, that breeding biomass was low and that management actions were required to reduce the catch by 50% (Fairclough *et al.* 2010). The management measures employed included limiting the overall commercial demersal wetline fishing effort, prohibiting commercial fishing in some areas, and imposing possession and size limits, and a closed season of two months for recreational fishing (Fairclough *et al.* 2010). To date, these

measures have been successful in reducing the estimated take of demersal species by the 50% required for their recovery. The recent assessment of the WCDSIMF indicated there were early signs of recovery in the dhufish stocks (Department of Fisheries 2013).

1.3 Recruitment monitoring

Regular monitoring of dhufish age structure and results of catch curve analyses have revealed that, particularly towards the southern end of its distribution, dhufish exhibit variable annual recruitment. The ability to monitor trends in the recruitment of juvenile dhufish could potentially be valuable for informing assessments of this species, e.g. as part of a “weight-of-evidence” assessment and/or for informing a fisheries integrated statistical model. Such an index of annual recruitment would have the potential to be used to forecast the strength of recruitment to the fishery which, if necessary, could enable pre-emptive management decisions to be made. However, for such an index to be used with confidence, it is first necessary to establish that trends in the index of juvenile recruitment are strongly correlated with trends in year class strength of adult fish, as observed in age composition samples. Thus, the first step is to trial potential methods over a period for their ability to detect variation in juvenile recruitment strength, over a period of time. Ideally, any recruitment monitoring would also cover different areas of the fishery to allow for spatial differences in recruitment throughout the distribution of the species.

The limited sampling undertaken during the first field component of the project in 2011 identified methods other than trawling, such as small fish traps and baited remote underwater video (BRUV), which may be suitable for monitoring dhufish recruitment. The report and photographic evidence of numerous juvenile dhufish on an abalone aquaculture lease of artificial habitat off Augusta also provided evidence to suggest that establishing artificial habitats in specific areas of habitat has potential for monitoring dhufish recruitment strength. However, as trawling was the only previously successful method for sampling small juvenile dhufish, it was important that alternative sampling methods be compared with the results obtained from trawling.

1.4 Objectives

The objectives for the additional year of field sampling covered in this report were to;

1. Regularly monitor, monthly if possible, the juvenile dhufish at an abalone lease site identified as harbouring dhufish off Augusta with stereo underwater video systems to detect changes in abundance, size and behaviour.
2. Trial the use of artificial habitats as a method for monitoring dhufish recruitment in the Perth metropolitan area.
3. Apply suitable survey methods to assess annual variation in juvenile dhufish abundance within the Perth metropolitan trawl area.
4. Investigate additional sites identified in south-western WA as potential nursery areas for juvenile dhufish, including established artificial habitats.
5. Assess the diet of the juvenile dhufish using traditional gut content analyses and molecular sequencing techniques to determine if prey habitat associations can be established.

2.0 General methods

The general details for survey methods which are common to a number of sections of this report are outlined below, with more specific details on the methods used are given in each section.

2.1 Stereo diver operated video (sDOV)

Diver operated stereo underwater video systems (sDOVs) are used to survey both benthic habitats and fish communities. This method has advantages over other survey methods such as underwater visual census techniques (UVC) for providing more accurate length estimates, a measurable sampling area, results that are less influenced by operator experience, and a permanent record of the survey which can be reviewed repeatedly. However, as with any video or visual technique, the results are influenced by water visibility at the time of the survey and video resolution with not all individuals being identifiable from the video footage alone. Thus, the processing of the survey video by the diver operator is recommended as this would result in a better interpretation of the survey and fish identification data. The method is also not effective for all species, particularly those which exhibit diver avoidance behaviours like large predatory species such as snapper (Willis *et al.* 2000).

The sDOV units consisted of two high definition video cameras (Canon HV 20 or Legria HFG10) in underwater housings mounted horizontally 450 mm apart on a base bar each inwardly converging at 4.0 degrees (Figure 2.1). The units were also equipped with a synchronising diode mounted on a bar in the front within the field of view of both cameras for processing the stereo footage. Each unit was calibrated using standard methods, both before and after use in a survey, to ensure the accuracy of length estimates.



Figure 2.1 Image of sDOV in use illustrating size, position of synchronising diode and positioning of inwardly converging cameras in housings.

The sDOV surveys generally consisted of belt transects covering the full extent of the site or star search patterns on the cardinal compass bearings (i.e. North, South, East & West) from a central marker point. In each survey type, the swimming speed and depth above seabed were generally constant at 0.2 m/sec and 0.5 m respectively. However, some deviations, and panning and pausing of the transects were required in order to obtain the best footage for estimating the numbers of juvenile dhufish in a large school or to increase the precision of length estimates by waiting for individual fish to be side on and/or close to the sDOV unit.

2.2 Baited remote underwater video (BRUV)

Baited remote underwater video systems are becoming routinely used to assess fish community composition in particular areas and habitats. The development of stereo BRUV systems has added the ability to estimate the lengths of most fish observed, allowing further assessment of size structure of species between areas (Watson *et al.* 2009).

The sBRUV units consisted of two high definition video cameras (Canon HV 20) in underwater housings, mounted horizontally 700 mm apart on a base bar each inwardly converging at 8 degrees. The units were also equipped with a synchronising diode for processing of the stereo footage and a bait pole with plastic coated wire mesh bait basket positioned 1.2 m from the cameras. Each unit was baited with approximately 500g of pilchards, deployed and left for the full 60 minutes of recording available in high definition progressive scan mode. Where possible, the units were separated by at least 200 m to avoid overlap of bait plumes. The units were calibrated using standard methods both before and after use in a survey to ensure accuracy of length estimates. The method has proven effective for assessing the relative abundance of fish species that are attracted to bait, such as carnivores and omnivores, but less so for herbivorous and planktivorous species.

2.3 Towed live feed underwater video

Towed live feed underwater video systems with GPS overlay are regularly used in benthic habitat surveys to assist with creating habitat distribution maps and ground truthing acoustic surveys. The system has also been used to provide fish species habitat association data by recording the habitat types occupied by particular species observed.

The general towed underwater video setup is described in Lewis *et al.* (2012) and consists of a live feed video camera in underwater housing connected by cable providing power and receiving the video output to the surface. The video output is viewed and recorded at the topside case on the surface. Most systems used for benthic habitat surveys also have a GPS overlay to record the position of the vessel on the video footage. The towed underwater video system utilised was enhanced with a stereo BRUV crossbar and synchronising diode to collect high definition stereo footage. The high definition footage allowed the identification and even detection of fish species that could not be observed on the live feed underwater video footage. The stereo footage was also important to provide an estimate of the size of each dhufish observed.

The towed video surveys were generally conducted as drift surveys on the smaller research vessel (*RV Snipe II*) due to limited manoeuvrability, but as towed surveys on the larger research boat (*RV Naturaliste*). For the smaller vessel setup the live feed video was attached to the BRUV crossbar with a tailfin to ensure setup pointed forward in the direction of the drift (Figure 2.2). The video setup was suspended from a bridle rope attached to each of the outside edges of the BRUV crossbar and a dive weight attached to the diode arm to angle the unit slightly

downwards. For the larger vessel the BRUV crossbar was mounted on the towed underwater video downweight used in previous surveys (Lewis *et al.* 2012).



Figure 2.2 Towed underwater video setup utilised on the smaller research boat where A- synchronising diode, B- live feed video camera, C- BRUV crossbar with stereo video housings, D- live feed video cable, E- GPS overlay antennae, F- topside case with monitor, video out plug and GPS overlay and G- 12v power supply .

2.4 Video processing

The sDOV, sBRUV and towed stereo video collected by the above methods were processed using the Eventmeasure software (SeaGIS Pty Ltd). Initially the videos were processed by adding point information for each species of fish observed, i.e. species, number on a single frame (MaxN), location/habitat (section of lease or reef), and behaviour (solitary or school). The second phase of processing was to obtain a length estimate for each juvenile dhufish, where possible. To keep track of individuals, each juvenile dhufish was allocated a number and tracked through time allowing repeated length estimates for each individual to be made. When schools of juvenile dhufish were encountered the processing became more complicated, see section 3.0. For each length estimate the Eventmeasure software gives a precision value. The precision is the average of the standard deviations for the X, Y and Z coordinates of the two measurement points which are calculated from the camera properties, three-dimensional intersection geometry and an image measurement precision of 1 pixel. For each individual with multiple length estimates the estimates were selected to allow for the swimming motion of the fish changing its measurable length and precision of the estimates. The largest length estimate, that would be closest to the fish's actual straight length without curvature due to swimming motion, with a high precision, ie precision value of <5mm, were used as the final length.

The live feed video from the towed underwater video system was processed separately by stopping the video every 30 seconds or at any changes in habitat types and recording the habitat type along the GPS coordinates from the GPS overlay. The towed video benthic habitat and positional data was imported into GIS mapping software (Arcview 10.0) to display the confirmed habitat types in relation to the sampling by other methods or sidescan sonar mapping of the area.

3.0 Surveys for juvenile dhufish

P. Lewis

3.1 Augusta site

The report and video of a number of juvenile dhufish at a recently established abalone aquaculture lease off Augusta in south western WA, received during the first phase of the project, presented a unique opportunity to collect information on the behaviour, seasonal abundance and growth of juvenile dhufish. The details of the artificial habitat types and natural habitats in the immediate area surrounding the site, along with the tests of different survey methods, could potentially lead to the development of a suitable regime for monitoring annual dhufish recruitment. The site was regularly surveyed for one year utilising sDOV.

The information collected during the previous phase of the project confirmed that juvenile dhufish are not cryptic and active during the day (Lewis *et al.* 2012). This behaviour allowed them to be effectively monitored by diver surveys. sDOV was utilised in the surveys to retain a permanent record of the surveys, record numbers of juvenile dhufish at the site, and also provide estimates of lengths of individuals and thus also potentially information on the year class cohorts and growth of juvenile dhufish at the site. The sDOV surveys also allowed the abundance of other species at the newly established artificial abalone habitat to be recorded and documented.

3.1.1 Methods

Location

The study area consisted of three sites located in Flinders Bay on the south west coast of WA near the town of Augusta (Figure 3.1). The sites are abalone aquaculture leases where various types of artificial habitat have been placed for grow out or sea ranching of hatchery produced abalone. The main lease site is approximately 3.8 km offshore in a water depth of 19 m. The secondary lease sites are located to the northwest of the main site in water depths of 16 and 14 m.

The main lease was established in January 2011 and initially consisted of six pads (cement railway sleepers with masonry blocks on top) but by August 2011, a range of the artificial habitats were in place (seven different artificial habitats in nine sections/configurations) spread over a distance of ~ 250 m and an area of ~ 2500 m² (Figure 3.2, Table 2.1). Thus, the sDOV survey was structured according to include these nine sections, and the fish observed on each section were recorded separately. The secondary lease sites also have a mixture of the seven different artificial habitat types spread over an area of ~ 900 m² (but with a lower number n<5 of each habitat types).

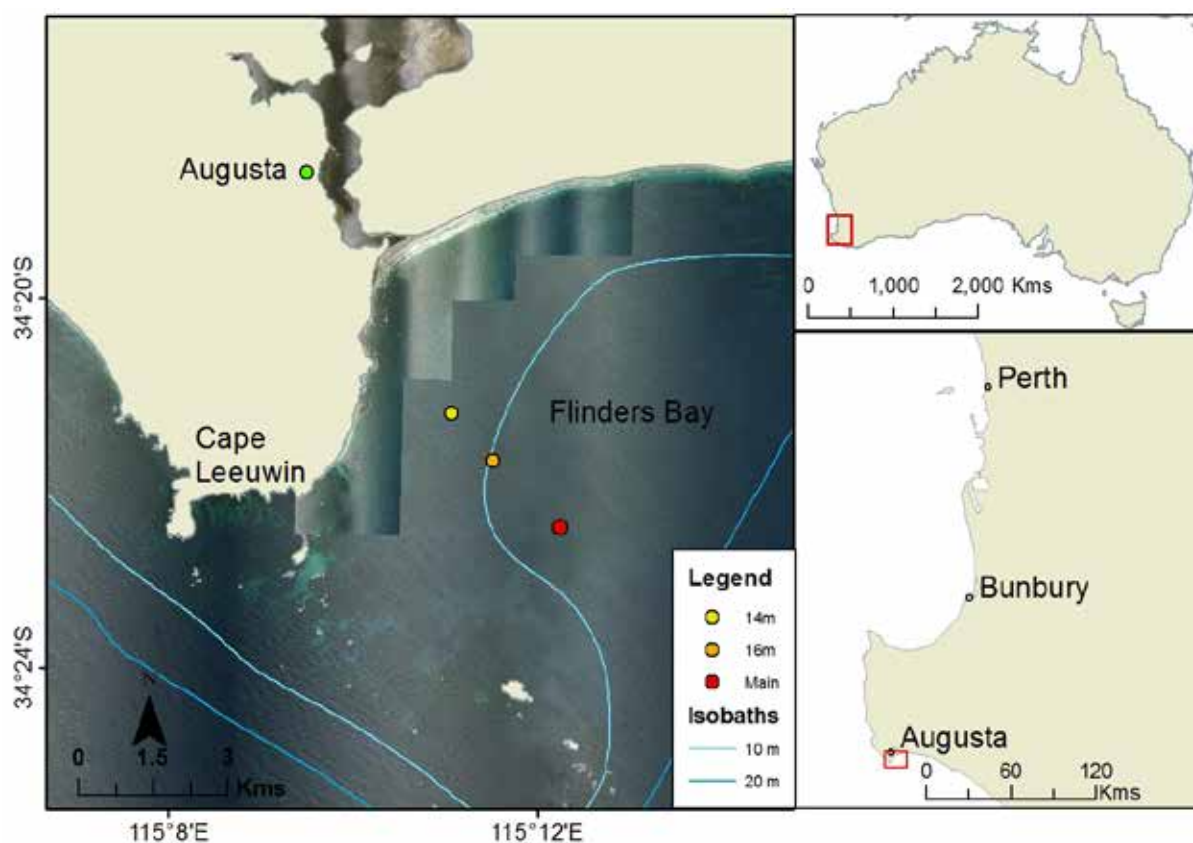


Figure 3.1 Location of study sites within Flinders Bay indicating depth and aerial photography showing lighter areas of sand and darker areas of seagrass or reef.

Table 3.1 Sections of artificial habitat along main lease site from east to west with the type, dimensions, configuration, No. of units and benthic habitat type.

Section	Type habitat	Dimensions (WxLxH)	Configuration	Units	Benthic habitat
Star blocks	Custom star shaped blocks	300 diam x 400mm	Individual	20	Sand
V pipes	Sewer pipes	375 diam x 1.5m long	V shape of 30 pipes	1	Sand
3 pipes	Sewer pipes	375 diam x 1.5m long	Groups of 3 pipes	8	Sand
Hollow blocks	Hollow concrete	400x400x400mm	Groups of 4 blocks	10	Sand
Solid blocks	Solid concrete	400x400x400mm	Groups of 4 blocks	10	Sand/ seagrass
Pads	Cement pad with bricks	1200x1200x 250mm	Individual units	6	Sand/ seagrass
Gutters	Kerbing	400x100x100 mm	V pattern of 10 blocks	10	Sand/ seagrass
Blocks west	Hollow and solid concrete	400x400x400mm	Continuous line of 40 blocks	1	Sand/ seagrass
Bricks west	Masonry bricks	260x400x260mm	Clusters of 3-4 bricks	20	Sand/ seagrass

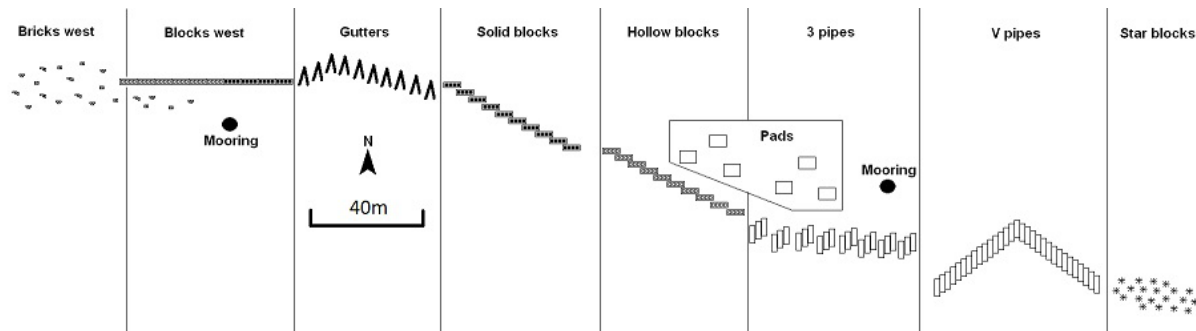


Figure 3.2 Diagram of main lease indicating the nine different sections and approximate orientation. Note: approximate scale.

Survey methods

The leaseholder and research divers conducted initial surveys utilising various underwater cameras to record images and footage of the juvenile dhufish and other fish species at the main lease. The nine sDOV surveys were conducted every four to six weeks from November 2011 onwards (Table 3.2). The sDOV surveys were carried out as modified free swimming or roving belt transects from one end of the lease to the other. The surveys were not always continuous as some diversions, pauses and panning were required to survey the six pads situated to the north, and obtain the optimum footage for precise length and abundance estimates of the juvenile dhufish when large schools were encountered or when solitary individuals were not in a measurable position when first observed. Over the year three different sDOV units, each with the same base separation, were used to carry out the surveys (Table 3.2).

The secondary lease sites were only surveyed on four occasions in 2012 by sDOV (Table 3.2). As these sites were spread over an area and not in a continuous structure star searches were conducted along the four cardinal bearings (North, East, South, and West) from the central mooring to cover most of the artificial habitats. As with the main lease site, the surveys were not always continuous with some pauses, diversions and panning to obtain the optimum footage of juvenile dhufish for length estimation.

Table 3.2 Timeline of surveys completed at main lease site and information obtained from each.

Date	Site/s surveyed	Survey type	sDOV unit	Abundance	Lengths
15-Mar-11	Main only	Diver survey		Y	N
5-Apr-2011	Main only	Diver video		Y	N
12-May-2011	Main only	Diver video		Y	N
12-Aug-2011	Main only	Diver video		Y	N
23-Nov-11	Main only	sDOV	Biodiversity1	Y	Y
17-Jan-12	All sites	sDOV	Biodiversity1	Y	Y
17-Feb-12	Main only	sDOV	Finfish	Y	Y
28-Mar-12	All sites	sDOV	Biodiversity1	Y	Y
10-May-12	Main only	sDOV	Finfish	Y	Y
7-Jun-12	Main only	sDOV	Finfish	Y	Y
6-Jul-12	Main only	sDOV	Finfish	Y	N
19-Aug-12	All sites	sDOV	Biodiversity2	Y	Y
18-Oct-12	All sites	sDOV	Biodiversity2	Y	Y

Processing

The sDOV footage was processed using the Eventmeasure 3.32 software (SeaGIS Pty Ltd). As each individual or school of fish was observed, the species, number, section of transect and behaviour (schooling in groups $n > 5$, small groups $n < 5$, or solitary) were all recorded. Where possible, each dhufish encountered was measured on a number of occasions to obtain the best estimate of their length, i.e. the estimate with the highest precision. Generally this was obtained when the individual was close to the sDOV and orientated side on. Each individual dhufish encountered in each section of the lease was allocated an identifying number.

Estimation of lengths for each individual juvenile dhufish was difficult when large schools were encountered. Precise length estimates of all individuals were not always possible at the time of MaxN i.e. the time when most number of individuals of a species is visible on the screen, due to some individuals being too far from the sDOV or not in the best position for accurate length estimation ie not side on to DOV. As a result, a period of ~ 3 minutes was identified when the majority of the school could be measured with the best precision. To identify each individual juvenile dhufish in a school and ensure none were counted more than once, each fish was allocated an individual number and tracked through the measurement period by stepping through the footage by five frames at a time. Many individuals were measured more than once and this not only helped with tracking of each individual but also ensured the most precise length estimates could be obtained, see section 2.4. During this measurement period a number of individuals left and entered the field of view or were hidden from view and only those which had not been measured before were allocated a number and measured. The secondary sites were processed in a similar manner recording the species, number, behaviour and artificial habitat type each was associated with.

Analysis

Juvenile dhufish

The overall abundance of juvenile dhufish at the main lease was compared through time for change due to emigration, mortality or new recruitment. Their abundance on each section of the lease was also examined to determine any preference for type of artificial habitat. The overall average length of juvenile dhufish was estimated for each survey and compared between surveys for change and to estimate the growth rate (in mm/day) for juvenile dhufish. The length frequency observed in each month for the main lease and in each section of the lease were also plotted to examine the changes in size structure due to growth, recruitment, emigration and mortality.

Initially, the growth of juvenile dhufish was estimated from the mean length of juveniles in each month and fitting a regression equation. However, due to emigration from the lease site this was not possible for the entire study period. Subsequently, each individual was allocated an age based on the assumed birthdate of February 1. The resulting length at age data was plotted and a linear regression fitted by least squares routine using the MX Excel solver add-in. As the larger and smaller age groups showed evidence of truncation due to immigration and emigration a linear regression was fitted to the length at age data for the 10-16 month age groups to give an alternate estimate of growth without the influence of emigration and immigration recorded at the site.

Observed changes in behaviour of juvenile dhufish with size, from fish mainly being solitary or occurring in small groups (< 5 individuals) to occurring in schools of > 10 individuals were analysed by comparing the number and size of juvenile dhufish in each behavioural grouping for each survey.

Other species

Changes in diversity and abundance of fish species were examined (i.e. by plotting) over time, both by including and not including the abundance of schooling species such as rough bullseyes (*Pempheris klunzingeri*) or fusilier sweep (*Caesiocorpius theagenes*), the presence of which highly influenced results.

The change in fish community composition between surveys was examined employing non-metric multi-dimensional scaling (nMDS) ordination, using Primer 6.0 (Primer E). The data were first logn-1 transformed to reduce the influence of schools and the Bray-Curtis similarity coefficient matrix calculated. The nMDS plots were generated employing Pearson similarity correlations, added at the significance of 0.8 to examine the key species differences driving the changes observed in the community structure.

The occurrence of breaksea cod (*Epinephelides armatus*) and Rankin cod (*Epinephelus multinotatus*) over time at the main lease through time allowed the growth rates of these species to be estimated from monthly changes in their estimated lengths. As with juvenile dhufish, when a number of length estimates were made for each individual in each month, the estimate with the highest precision was used. In addition, the mean of the 3 most precise length estimates was also calculated for each individual in each survey. The estimated lengths for each fish were plotted over time and linear regression analysis conducted to assess the growth rate along with the overall change in length over time. In addition, changes in the colouration of the juvenile Rankin cod with respect to size was documented.

Additional analyses

The effectiveness of the sDOV technique for juvenile dhufish was assessed by determining the percentage of juveniles observed that were measured (overall and for each survey), the range from the sDOV (for each metre away) at which the juveniles were measured and the precision of the length estimates (as a % of the length estimate).

3.1.2 Results

Main lease

Juvenile dhufish abundance

A total of 684 juvenile dhufish were counted on the main lease site over the 13 surveys. The maximum abundance recorded on the lease was 144 in January 2012 and the highest abundance in one section was 127 around the V-pipes in the November 2011 survey (Figure 3.3). This high abundance occurred four months after the deployment of the main proportion of artificial habitat in July 2012 and remained high during the January 2012 survey after which abundance declined rapidly, until June 2012 when there were 36 juveniles recorded. After this time the abundance continued to decline slowly to a minimum 25 juveniles in the October 2012 survey. The abundances recorded before and immediately after the deployment of the majority of artificial habitat in July 2011 are not directly comparable but are given to indicate the timing of first appearance in March 2011. These illustrate that the abundance of the small juveniles increased gradually from two to 12 individuals on the initial small area consisting only of the pads section of artificial habitat.

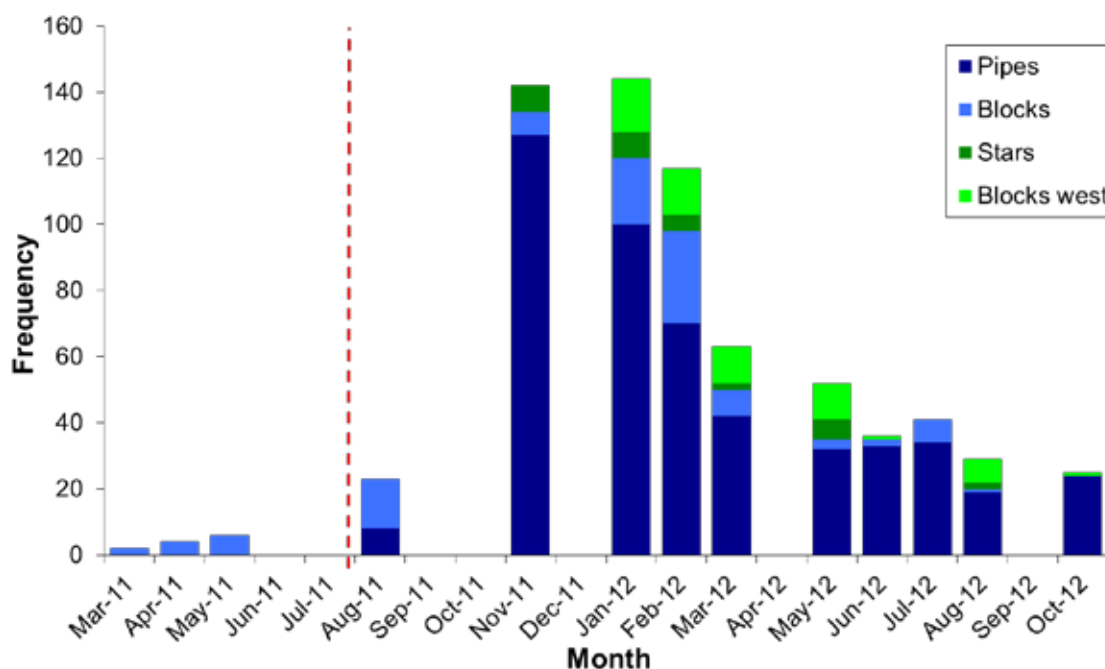


Figure 3.3 Abundance of juvenile dhufish at main OGA lease site by month during study period indicating abundance in each section, note V-pipes and 3-pipes grouped as pipes and hollow blocks and solid blocks grouped as blocks. Red line indicates time for deployment of the majority of the artificial habitat.

Preference for sections/types of artificial habitat

Juvenile dhufish were recorded on all sections of the lease except around the gutters where no juveniles were recorded in any survey. On each sDOV survey, the majority (60-96 %) of juvenile dhufish were recorded in the region of the V pipes and 3-pipes sections of the lease (Figure 3.3). Small numbers of juveniles ($n < 30$) were recorded on other sections of the lease, particularly in the solid and hollow blocks sections until the October 2012 survey when only a single juvenile was recorded in the blocks west section.

The juveniles in the pipes region of the lease occurred as a single school or a number of discrete schools on every sDOV survey. During the November and January surveys there were three distinct dhufish schools, each with ~ 30 individuals, in the area of the V pipes which merged into one large school of 124 on the November survey (Figure 3.4).



Figure 3.4 Image of part of juvenile dhufish school (n=79) recorded at V-pipes section of main lease in November 2011.

A school of juvenile dhufish (n=14) observed in the western blocks section was the only other dhufish school recorded. This school of juveniles was mixed with a large school of over 100 rough bullseyes (*Pempheris klunzingeri*) from February to May 2012. Prior to this in January 2012, there were 16 scattered solitary individuals encountered in this section of the lease and after the formation of the school there were no solitary juveniles in the western section.

All of the smallest juveniles of TL<100 mm (n=5) were associated with the smaller masonry blocks on the pads or scattered around the lease (Figure 3.5). The small juveniles were always closely associated with some form of refuge and were regularly observed inside the hollows of the masonry blocks and rarely ventured more than one or two metres from some form of artificial or natural refuge.



Figure 3.5 Image of two very small juvenile dhufish associated with masonry block on main lease in May 2011.

Size

It was possible to estimate the total length for 425 juvenile dhufish. The estimated lengths ranged from 88 – 246 mm. The mean length of juvenile dhufish for each survey initially increased over time from 132 mm in November 2011 to 198 mm in May 2012 (Figure 3.6), even though the numbers at the site were steadily decreasing (Figure 3.3). After the May 2012, survey there was a steady decrease in mean length and increase in the standard error due to bi-modality of the data.

During the initial surveys from March – August 2011, very small juveniles associated with the masonry blocks on sand and on the pads were recorded. However, as these were not sDOV surveys, the sizes of these juveniles could not be accurately estimated. Their small size could be gauged by comparison to the known width and height of the blocks, which is 280 mm (Figure 3.5). Most of these juvenile dhufish were less than 1/3 the width making them less than 85 mm in TL.

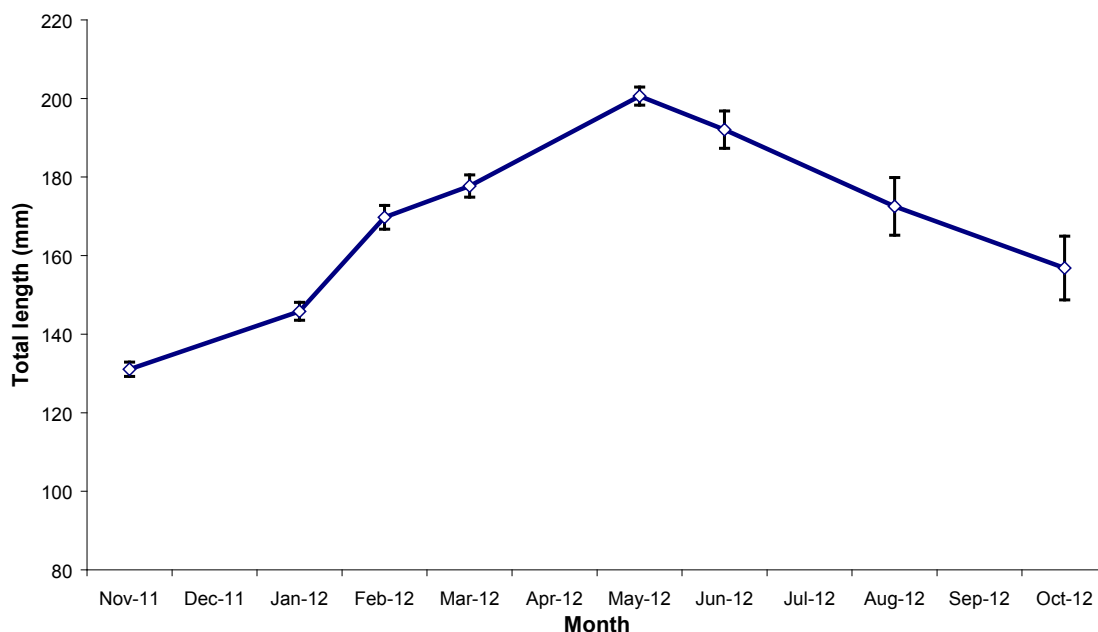


Figure 3.6 Average length of juvenile dhufish recorded at the main lease in each survey. Error bars represent 1 standard error.

The monthly length frequency (Figure 3.7) illustrates why the average length declined after May 2012 with the appearance of smaller juveniles (TL<160mm) after this time and the continued emigration of the larger juveniles (TL>200 mm). The initial year class measured in November 2011 ranged in length from 80-180 mm in TL and would likely be approaching 1 year of age, as spawning occurs primarily in the summer months so spawned in 2010/11. The smaller size classes recorded from June-October 2012 is likely to represent the following 2011/12 spawned year class. Within each year class there is a large range of sizes and there were several modes. After the May 2012 survey the larger mode in the 2010/11 spawned year class appeared to have mostly emigrated from the lease, see below.

The average size of juvenile dhufish occurring in small groups ($n < 5$) or as solitary individuals was significantly smaller than the size of juveniles occurring in schools (at $P < 0.05$) on the initial surveys and again in August 2012 when the following year class of juveniles were in sufficient numbers (Table 3.4). The single individual observed on the October 2012 survey was smaller than all of the individuals recorded in the school but this difference was not significant due to the small sample size.

Table 3.4 Summary of ANOVA analysis of difference in size of juvenile dhufish occurring as individuals Vs those in schools.

Survey	Sum of squares	F-value	P
Nov11	1469.38	4.444	0.0375
Jan12	5814.53	16.77	>0.001
Feb12	2583.8	6.71	0.011
Mar12	670.84	1.60	0.21
May12	43.75	0.15	0.70
Jun12	2423.26	3.80	0.061
Aug12	20836.18	27.34	>0.001
Oct12	3699.17	2.49	0.13

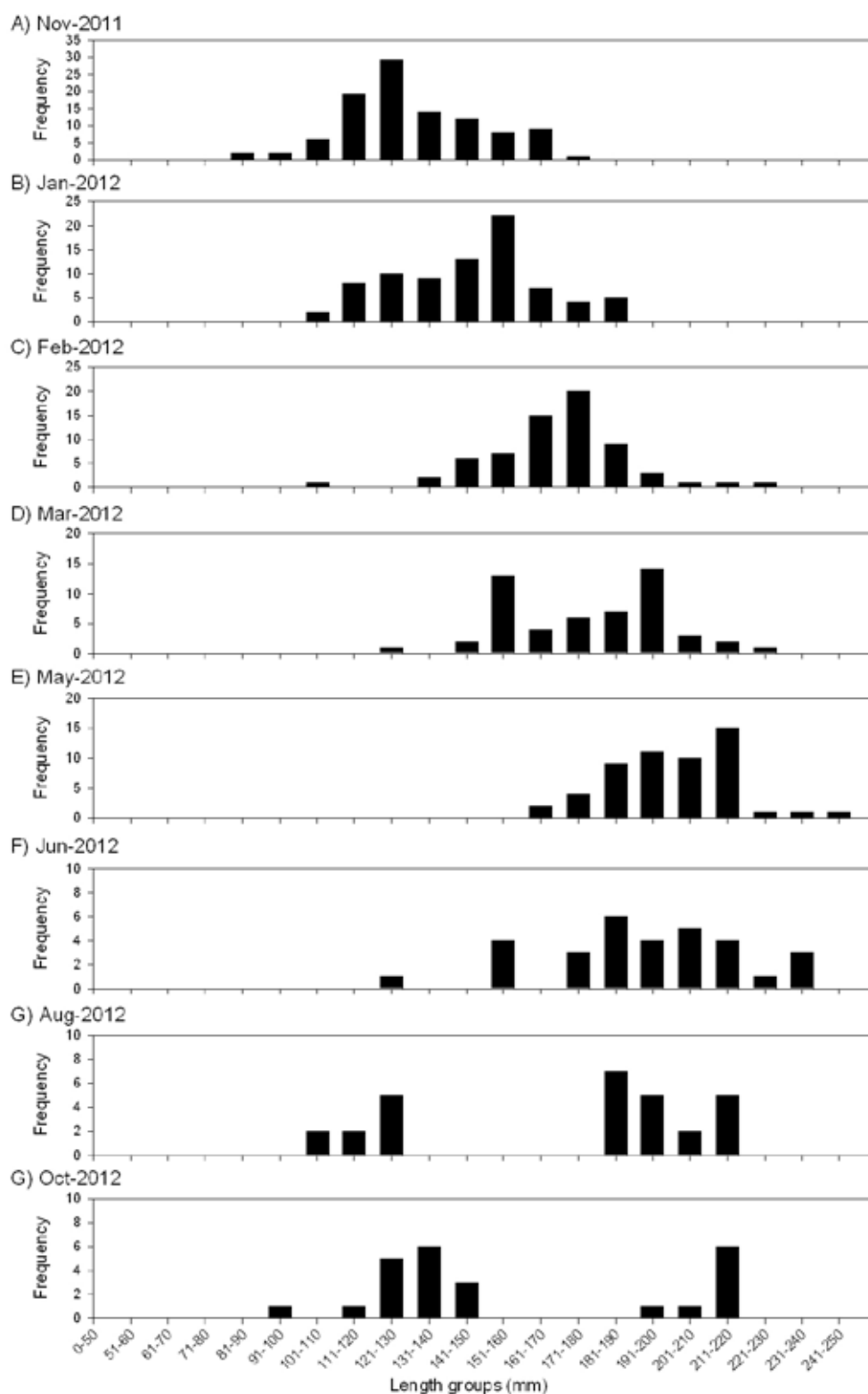


Figure 3.7 Monthly length frequency plots of juvenile dhufish at main lease site.

The change in abundance of each year class over time at the main lease is given in Figure 3.8. This clearly indicates the continual decline in the abundance of the 2010/11 spawned year class at the main lease towards the end of the study but also the gradual increase in the abundance of the 2011/12 spawned year class.

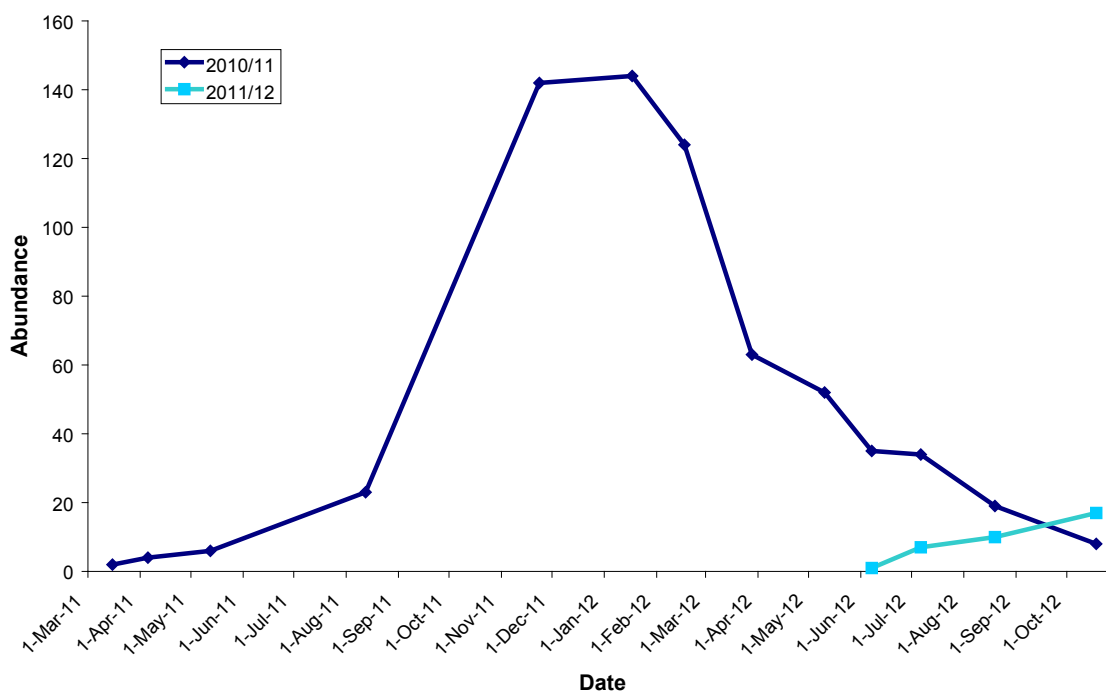


Figure 3.8 Abundance of each year class of juvenile dhufish at the main lease over time.

Growth

The growth rate estimated from the average lengths of juveniles from November 2011 to May 2012, before the appearance of the following year class (Figure 3.6) was 0.42 mm/day or 153mm/yr. The analysis of the length at age data resulted in an annual growth = 97.8 mm with an initial value of 55.4 mm (Sum Log likelihood= -1889) (Figure 3.9). The results are influenced by the immigration and emigration of juveniles to the site at 80-120 mm and 210-240 mm respectively. The slope of the 10- 16 month age groups (with little evidence of immigration or emigration) was 142.9 mm/yr, intercept at 9.8 mm ($R^2=0.9734$). Further analyses will be conducted to assess the sizes and ages of immigration and emigration to the site along with the growth rate once these influences are removed.

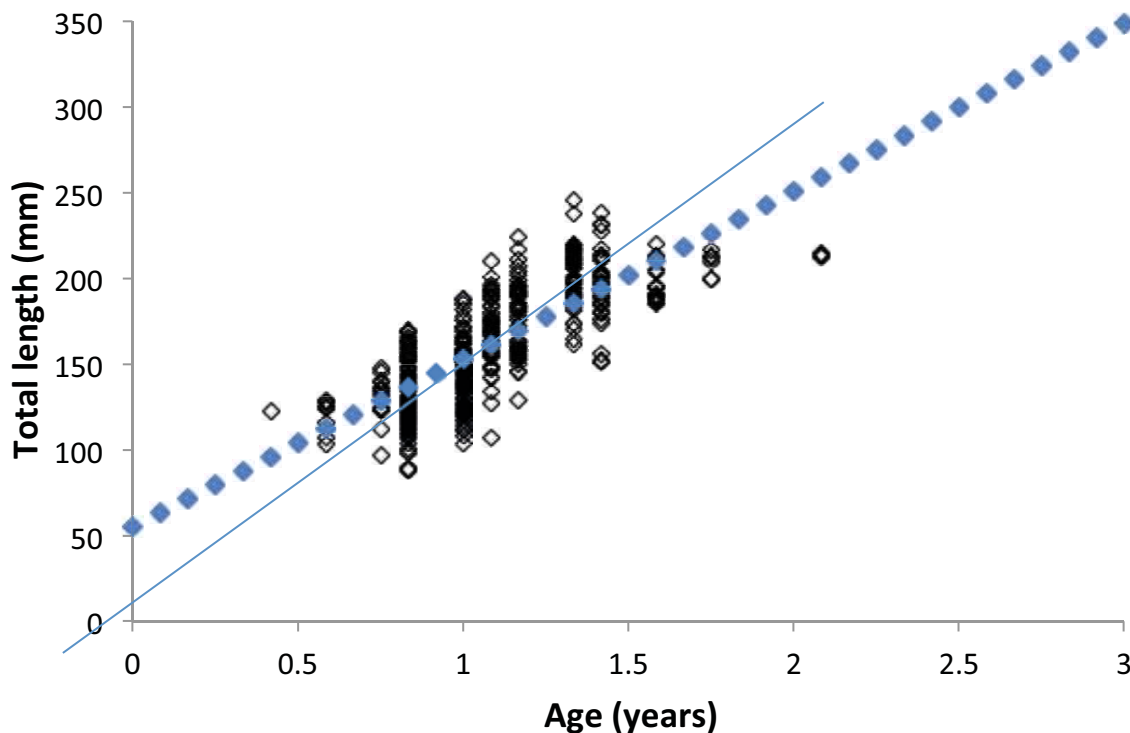


Figure 3.9 Length at age data for juvenile dhufish measured at the Augusta main lease between Nov2011 and Oct2012, where blue dots are estimates from overall regression equation (n= 425) and line is regression fitted to 10-16 month age groups only (n= 373).

Behaviour

Even though the schools of larger juveniles were always associated with hollow pipe sections, they were only occasionally observed inside or sought refuge inside pipes (Figure 3.4). They were regularly observed to move away as a school when approached by divers to an adjacent section of artificial habitat on the lease and rarely were observed entering hollow pipes. However, they may seek refuge inside when threatened by predators.

Over the course of the surveys and from many hours of observation of juvenile dhufish, it was apparent that some individuals displayed agonistic behaviour towards conspecifics. In general some of the smaller solitary juveniles appeared territorial, chasing away other small juveniles that ventured towards their refuge. Such agonistic behaviour was not as apparent in the juveniles that formed schools although over the course of the study a few larger individuals remained solitary and were aggressive towards their conspecifics. Such territorial behaviour may explain the solitary nature of the smaller juvenile dhufish (TL<130 mm) and may indicate that such refuges are in short supply and thus “defended”. It would also explain why these smaller sizes are rarely found in large numbers but as small groups. It is only when they grow to a slightly larger size of TL>130 mm that they appear to leave their refuges and form schools on areas of suitable habitat as also observed at the main lease.

Evidence that the solitary juveniles join the schools was obtained on two occasions a) March 2012 and b) August 2012. During each survey a number of solitary individual juvenile dhufish were observed throughout the lease while on subsequent surveys fewer solitary juveniles were recorded. Yet the numbers on the lease had not changed dramatically and it appeared that these solitary individuals had on occasions a) formed into a school at the western end of the lease away from the main school and b) joined the main school at the v-pipes section on these subsequent surveys.

Natural habitat associations

The benthic habitat surrounding the western section of the lease from the 3 pipes section onwards consisted of open flat sand with variable but often large amounts of mixed macroalgal wrack, particularly after large swell events (Figure 3.12). In comparison, beds of eelgrass (*Zostera* sp.) and a small section of paddleweed (*Halophila* sp.) occurred in varying densities amongst the sandy habitats surrounding the eastern section of the lease, from the hollow blocks onwards (Figure 3.13 and 3.14). Over the course of the study the artificial habitats have become extensively colonised by a range of macroalgae (Figure 3.12 and 3.16).



Figure 3.12 Benthic habitat of sand with macroalgal wrack in the eastern section of the main lease.



Figure 3.13 Benthic habitat of sand with eelgrass (*Zostera* sp.) and paddleweed (*Halophila* sp.) in the central section of the main lease.

Generally, the solitary smaller juvenile dhufish observed were associated with some form of refuge and would often utilise this when approached by divers. In most cases this refuge was in the form of the artificial habitats on the lease (Figure 3.4) but on a number of occasions the juveniles sought refuge in the surrounding natural habitat. On each occasion this natural refuge was in the form of the surrounding eelgrass beds in the western portion of the lease (Figure 3.14) or the macroalgal wrack in the eastern portion (Figure 3.15).



Figure 3.14 Image of a small solitary juvenile dhufish (TL<100 mm) associated with eelgrass beds in the western section of the main lease site.



Figure 3.15 Image of a small solitary juvenile dhufish (TL<100 mm) associated with macroalgal wrack in area surrounding the artificial reefs at Augusta main lease site.

Species associations

Over the nine sDOV surveys 48 species of fish were recorded on the main lease. The number of species observed on each survey increased from only 11 in November 2011 to 24 in July 2012 and remained at around this number on subsequent surveys (Figure 3.10). The higher overall number of species, particularly in latter surveys, indicates that many species were not recorded on every survey and may be only transient or seasonal at the site. Along with dhufish, only five other species, i.e. western king wrasse (*Coris auricularis*), black spotted wrasse (*Austrolabrus maculatus*), blue-spotted goatfish (*Upeneichthys vlamingii*), silverbelly (*Parequula melbournensis*) and snakeskin wrasse (*Eupetrichthys angustipes*), were recorded at the main lease site on all of the nine surveys.

Even though the diversity of fish species at the site increased over the survey period, the abundance was highly variable (Figure 3.10). Much of this variability was due to the influence of the large schools of rough bullseyes (*Pempheris multiradiata*) or fusilier sweep (*Caesiocorpius theagenes*), which were observed in varying numbers on some but not all surveys. The overall abundance, without these schooling species, decreased from 245 in November 2011 to 118 in May 2012 and remained stable at this level until the most recent survey in October 2012 when it increased to 224 individuals. The data revealed this was predominantly due to the increased abundance of western king wrasse at the site from October 2012.

During initial sDOV surveys (November, January and February), juvenile dhufish were the dominant species on the lease and even though their numbers declined, they remained the 2nd or 3rd most abundant of the non-schooling species for all surveys. The influence of their change in numbers on the community composition is evident in their appearance as a significantly correlated species in the nMDS plot of the fish community composition (Figure 3.11). As mentioned above, the schooling species (rough bullseye and fusilier sweep) were not observed on each survey and their influence on the fish community composition can also be observed in the nMDS results. The significantly correlated species can be divided into their habitat associations with the ‘sand associated species’ (*U. vlamingii*, and *Sillago* sp.) grouped together in the upper portion of earlier surveys (November, January and February) and the ‘reef associated species’ (*Chelmonops curiosus*, *Chrysophrys auratus*, *Anoplocapros lenticularis* and *Neatypus obliquus*) also grouped together in the lower portion of later surveys (May, June, July, August, October) as the artificial habitats become more established.

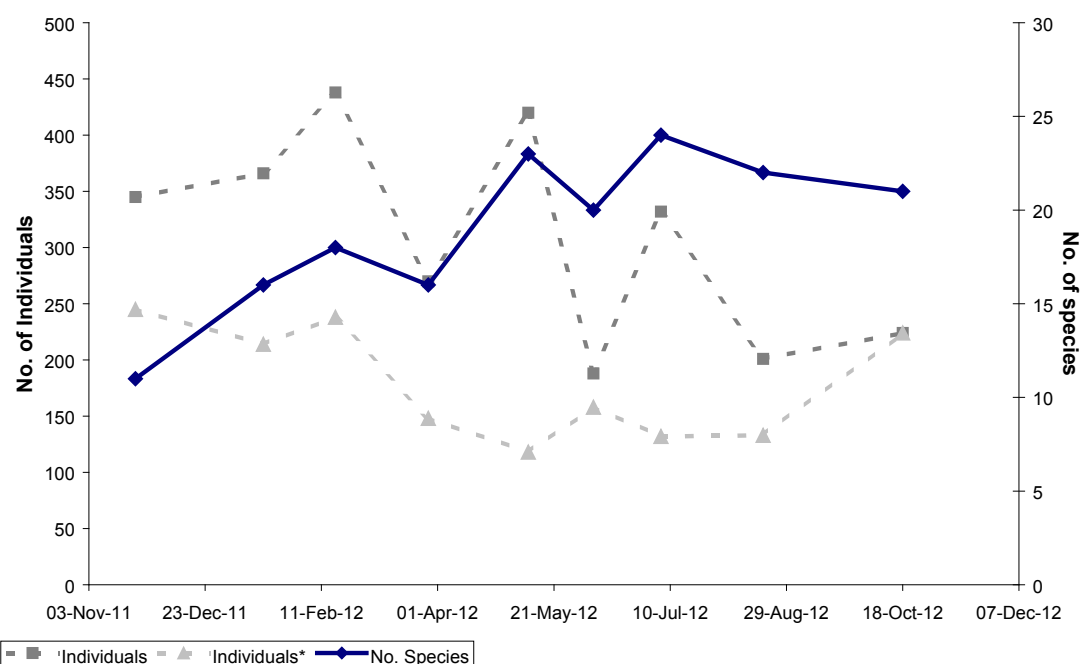


Figure 3.10 Timeline of diversity and abundance of species at the main lease site off Augusta where * denotes abundance without schooling species.

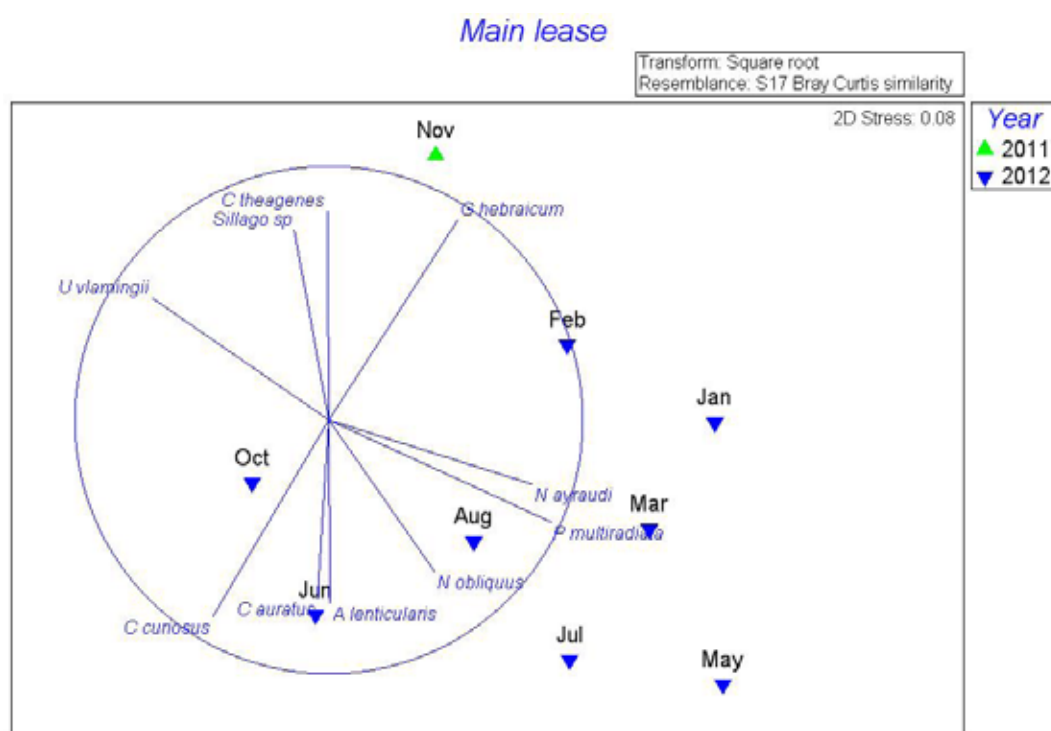


Figure 3.11 nMDS plot of fish community composition for each survey at main Augusta lease site, with Pearson correlations at significance of >0.8 where circle indicates full correlation.

Growth for individual breaksea cod and Rankin cod.

The regular observation of an individual breaksea cod and Rankin cod in the same section of the main lease on a number of consecutive surveys provided the opportunity to assess the growth of these individuals.

On every survey of the main lease, except February, an individual breaksea cod was observed in the vicinity of the V pipes section (Figure 3.16). The estimated total length of the breaksea cod increased linearly from 208 mm when first recorded in November 2011 to 288 mm in October 2012 (Figure 3.17). There was little difference between the length estimates from the most precise measurement to the average of the three most precise measurements. To achieve this growth of 80 mm in 11 months the average growth rate required is 0.24 mm/day.



Figure 3.16 Image of breaksea cod observed on V-pipes section of Augusta main lease.

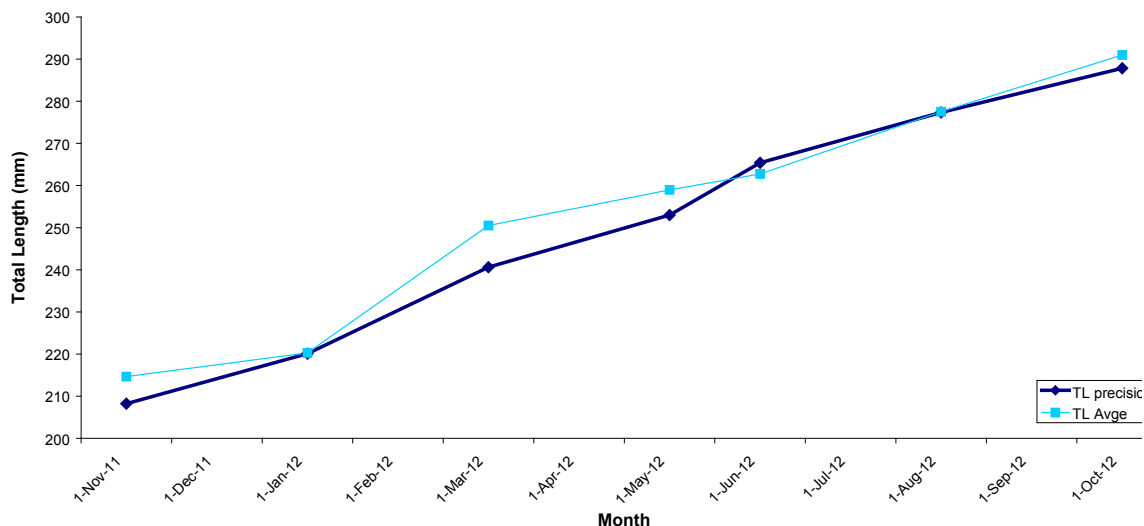


Figure 3.17 Change in time of averaged (light blue) and most precise (dark blue) total length estimates, for the individual breaksea cod recorded on each sDOV survey at the Augusta main lease site. Note, y-axis does not start from zero (0).

On each survey from February 2012 onwards a juvenile Rankin cod was observed in the vicinity of the 3 pipes section of the main lease (Figure 3.18). The colouration and appearance of this individual changed between and during surveys going from a pale background colouration with faintly discernable white spots to a dark background with distinct white spots (Figures 3.18 a,b). The colouration of the fin margins was also observed to change from light to darker colours (Figure 3.18c).

The estimated total length of the Rankin cod increased from 150 mm in February 2012 to 259 mm in October 2012 (Figure 3.19) with little difference between the methods of length estimation. To achieve this growth of 109 mm in eight months the average growth rate required is 0.45 mm/day.

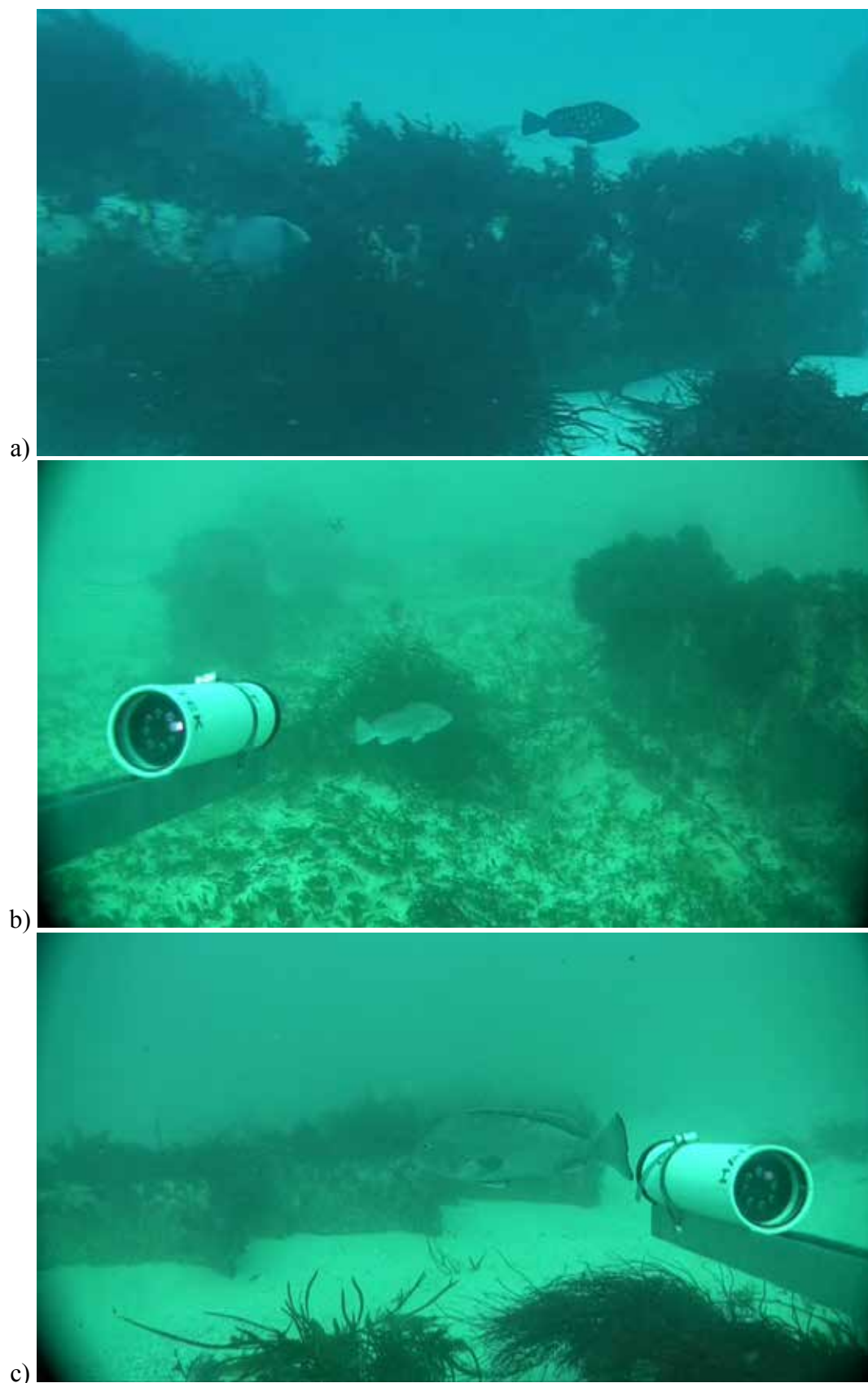


Figure 3.18 Image of a Rankin cod recorded on the main lease illustrating variations in appearance.

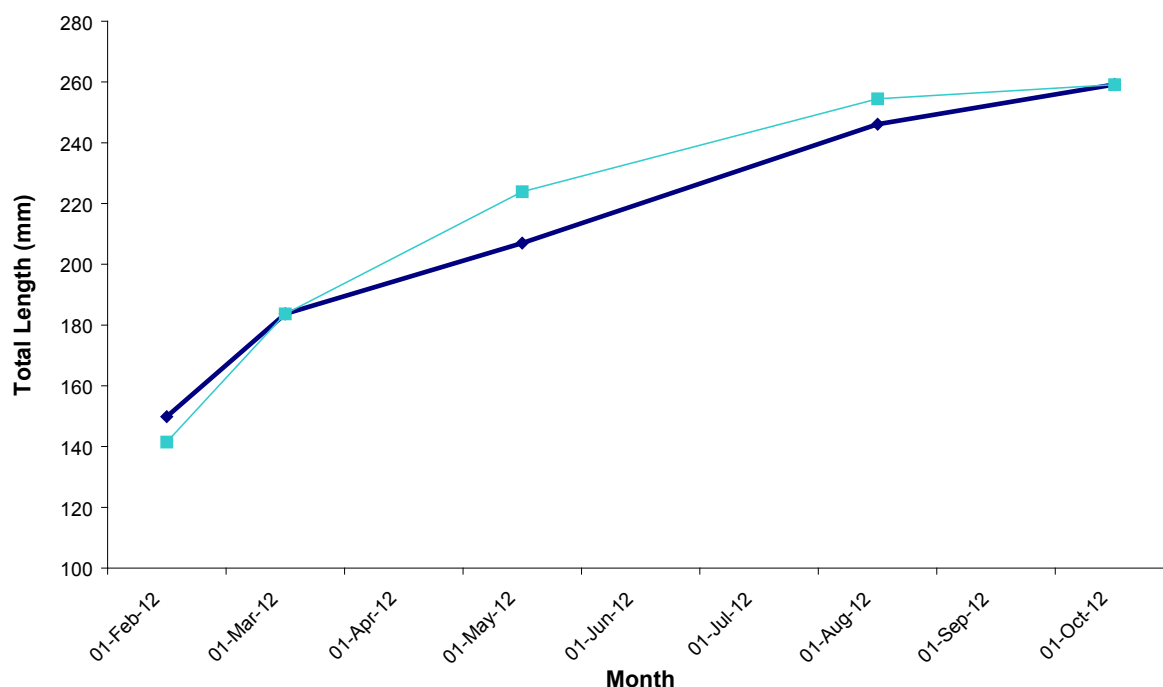


Figure 3.19 Change over time of averaged (light blue) and most precise (dark blue) estimated total lengths for the individual Rankin cod recorded at the Augusta main lease site sDOV surveys.

Inshore leases

The surveys of the two inshore leases were less regular than the main lease, with only four sDOV surveys carried out in 2012. The abundance of juvenile dhufish at these sites was considerably lower than at the main lease ranging from none, at either site in the October survey, to five individuals on each site.

On all but one occasion the juvenile dhufish observed were recorded as solitary individuals associated with pipes, blocks, bricks and the mooring. In the March 2012 survey of the 16 m site three of the juveniles formed a small group associated with an area of masonry blocks.

The estimated sizes of juveniles recorded at the inshore leases were similar to those at the main lease, ranging from 90-220 mm in TL (Figure 3.20). The change in abundance and irregularity of the surveys did not allow an estimate of juvenile dhufish growth rate to be determined for these sites. The 2010/11 and 2011/12 spawned year classes were both observed on the shallower 14 m site but the 2011/12 year class were not recorded at the 16 m site.

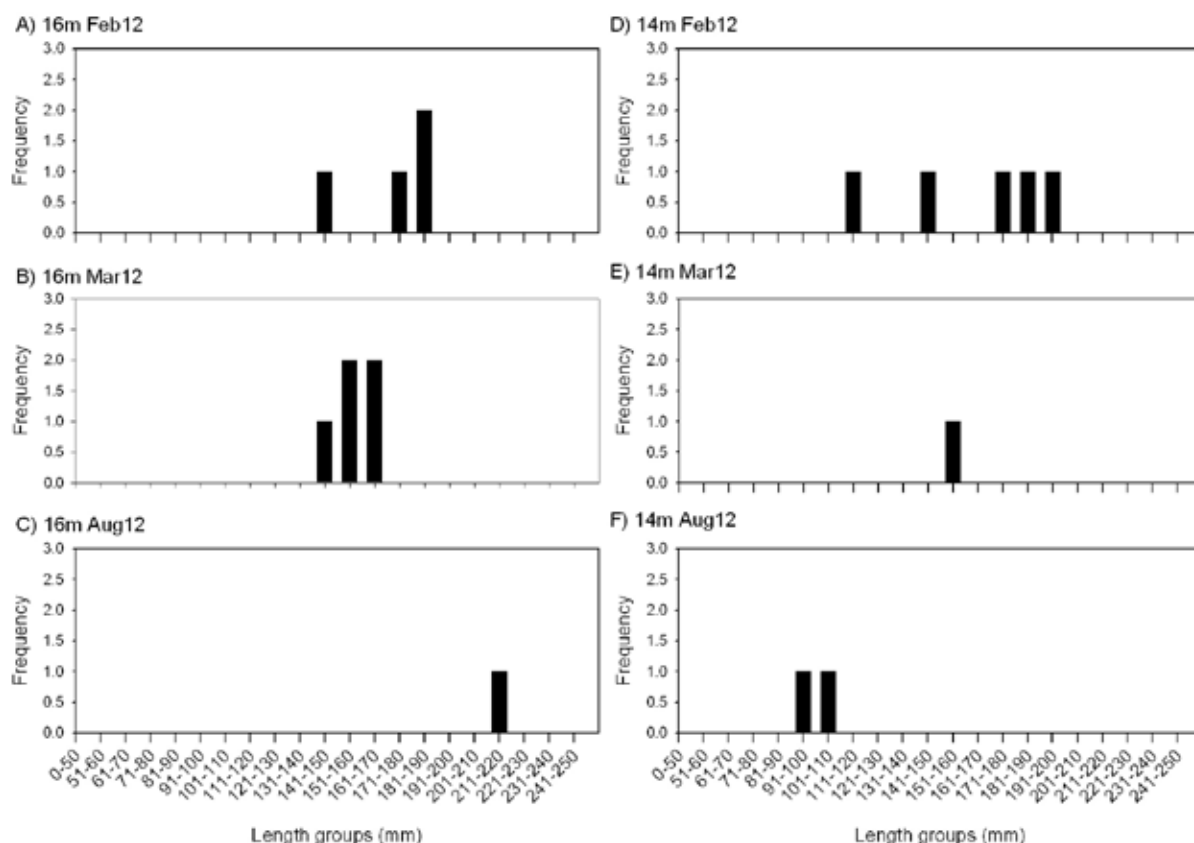


Figure 3.20 Monthly length frequency plots for juvenile dhufish recorded at the secondary lease sites.

The habitat surrounding the inshore sites differed between the two sites and to that at the main lease consisting of more seagrass beds. The 16 m site had a mixture of dense seagrass beds (*Amphibolis* sp.), with some sparse seagrass (*Posidonia* sp.), paddleweed (*Halophila* sp.) and eelgrass (*Zostera* sp.) in the sandy areas (Figure 3.21). The 14 m site in comparison had only sparse seagrass (*Posidonia* sp.) in the form of tussocks (Figure 3.22). The smaller juveniles were observed utilising this surrounding habitat as a refuge. On a number of occasions juvenile dhufish were recorded utilising the seagrass tussocks as a form of refuge (Figure 3.21).



Figure 3.21 Image of benthic habitat in area of 16 m site where longer thicker strands are *Posidonia* sp. seagrass, finer strands are *Zostera* sp. eelgrass and dense beds are *Amphibolis* sp. seagrass.



Figure 3.22 Image of an early recruiting juvenile dhufish (TL < 100 mm) associated with *Posidonia* sp. seagrass tussock in area surrounding the artificial habitat at the 14 m site.

The overall number of fish species recorded at the 14 and 16 m sites were only 17 and 24 species, respectively. The maximum diversity on a survey was only 10 at the 14m site and 14 at the 16m site. The lowest diversity at both sites was recorded in the Oct 12 survey when the visibility was poor, the swell was at 2.0 m and there was large amount of macroalgal wrack after a large swell event three days before. The most abundant and consistently recorded species at the 14m site were the rough bullseye and western king wrasse which also occurred in similar numbers at the 16m site along with schools of sea trumpeter (*Pelsartia humeralis*), longfinned pike (*Dinolestes lewini*) and old wives (*Enoplosus armatus*) on some but not all surveys.

Effectiveness of sDOV for juvenile dhufish

The sDOV technique proved very effective for monitoring the abundance and estimating the length of juvenile dhufish. The juveniles while wary of divers and moving off a short distance when approached did not generally take flight and disappear into the distance or leave the vicinity in which they were first observed. This allowed for them not to be repeat counted in different sections of the lease. Over the eight sDOV surveys 425 of the 615, or 69% of individual juvenile dhufish could be measured with confidence. On each of the surveys with more than 100 juveniles the percentage of the total number measured was between 55 and 70% due to not all fish in the schools being measurable. On other surveys with less than 100 the percentage was 95-100%. The suitability of the technique is also indicated by the range at which the juveniles were measured with 95% of lengths estimate for fish between 1 and 3.0 m from the sDOV. The effectiveness of the roving transect technique for estimating the lengths of juvenile dhufish is displayed in the precision of the estimated lengths with 87% of estimates having a precision of 10% or less.

The main reason it was only possible to estimate the total length for 69% of the juvenile dhufish observed was that when schools of more than 50 juveniles were encountered it was generally only possible to measure 50% of the individuals. Individuals could not be measured due poor orientation (facing away from sDOV), distance from the DOV or because they were obscured in one camera by other fish or macroalgae. The light colouration of the nose and tail of juvenile dhufish also meant measurements were not always possible for individuals against a sand background or when visibility was poor as both caused poor edge definition.

3.1.3 Discussion

The regular surveying of the artificial habitats on abalone lease sites off Augusta by diver stereo video has provided valuable information on the seasonal abundance, behaviour, habitat use, and growth of juvenile dhufish. The high abundance of over one hundred juveniles recorded at the main lease site, which has not been reported elsewhere and the successive year class recorded at this same site highlight the potential for the method and the site to be used to monitor the annual recruitment of dhufish. The occurrence of juveniles at all three of the artificial habitat sites in the area, which differed in depth and surrounding habitat, may also indicate the usefulness of artificial habitats in monitoring dhufish recruitment and the potential of suitable artificial habitats to enhance localised recruitment to a given area should both be investigated further. The regular sDOV survey technique proved to be effective for monitoring changes in size and abundance of juvenile dhufish as well as other fish species at these recently deployed artificial habitat sites. The results also indicate the suitability of the technique to monitor the growth, recruitment and range expansion of other species with the growth of breaksea cod and Rankin cod along with the range extension for the tropical Rankin cod to the Augusta area documented. However, there are still some gaps in the knowledge of juvenile dhufish with only a few individuals less than 90 mm in TL recorded.

The abundance of juvenile dhufish at the main Augusta abalone aquaculture lease site varied over the year and may have been due to seasonal fluctuations in abundance, predation, or behavioural changes in dhufish with respect to size resulting in the movement of juvenile dhufish from the surrounding areas to the artificial habitats and then subsequently to natural reefs. The high abundance of the 2010/11 cohort of juveniles in the 120-180 mm size class occurred at the site over a period of five months from November 2011 until February 2012. The abundance of the following 2011/12 spawned year class was still increasing at the site when the final October 2012 survey was completed indicating that their peak in abundance may subsequently occur over the

same period. Ideally any recruitment monitoring survey would be timed to coincide with the highest abundance of the dhufish recruits at the site. The optimised time of sampling to detect highest abundance and hence index for previous year is suggested to be between November and January when the highest abundances of new (approximately one year old) recruits were recorded.

The abundances of juvenile dhufish recorded at the inshore sites were dramatically lower than at the main site with a maximum of only four or five individuals recorded and no juveniles on the final survey. This may have been due to the smaller amount of artificial habitat at these sites with only approximately 1/10th of the artificial habitat in the area. The difference may also been due to the different surrounding habitat of predominantly *Posidonia* and *Amphibolis* seagrass beds and not sandy areas as surrounding the main lease site. Although at the start of the project finding a site with four or five juvenile dhufish would have been regarded as an outstanding result. Thus, the occurrence juvenile dhufish at these shallower sites of different benthic habitat types to the main lease may indicate the effectiveness of the artificial habitats for monitoring recruitment and the occurrence of juvenile dhufish in other areas of such habitat types along the coast.

It was observed that juvenile dhufish occurred at Augusta abalone lease site within one month of the artificial substrate being deployed and in high numbers soon after the additional artificial habitat was deployed in July 2011. On both occasions there was little time for the benthic community to become established on the new substrate which indicates the juveniles were predominantly using the site and artificial habitats for the refuge and not the food resources they provided. The smaller solitary juvenile dhufish at the Augusta sites regularly utilised the artificial habitats as a form of refuge when approached often entering the hollows of the masonry blocks or pipes. The juveniles were also observed to use some of the surrounding natural habitat as a refuge and this may indicate that they are naturally closely associated with fronds of seagrass, beds of eelgrass or patches or macroalgal wrack occurring in predominantly sandy habitats of the surrounding area.

The juvenile dhufish length frequency data collected at the main lease site clearly showed a wide range in the size of juveniles present in each survey. In each month the size of juvenile dhufish ranged over 80-100 mm in length. A similar range in size of juveniles for each month was recorded by Hesp *et al.* (2002) in marine waters near Perth. The range in sizes may reflect the extended spawning season of dhufish along the west coast (up to six months, but with most spawning occurring over 4 months) possibly resulting in a difference of up to 6 months in the ages of juveniles of a given year class. Such a difference in age would produce this range in size of each year class if recruits were arriving at the site throughout the spawning season.

The length frequencies and monthly average sizes of dhufish increased during each 4-6 week period between surveys. Even though the abundance of juveniles decreased from January 2012 onwards, the overall growth rate of juvenile dhufish from November 2011 to the May 2012 survey was determined to be approximately 98 mm/year for juveniles in the 100-240 mm size range. After this time, the emigration of the larger individuals and the immigration of the new year class led to a decrease in the monthly average size, and thus growth could not be estimated from the average size alone. This growth rate of the age groups not truncated by emigration and immigration was determined to be 143 mm/year. This estimate is in line with that determined by Hesp *et al.* (2002) for the growth rates of similar sized juvenile dhufish of approximately 120 mm per year.

Further examination of the monthly length frequency data revealed the wide range in sizes of over 70 mm in TL which could be tracked from survey to survey. The growth rates for the smaller and larger individuals were in line with the overall growth rates. The existence of such a wide range in the length frequency data may be due to the extended spawning season of dhufish contributing recruits of differing ages and corresponding sizes. Thus the larger juveniles may

have been spawned early in the season (November – December) and the smaller spawned later (March-April). The ability to distinguish modes within the 2010/11 spawned year class may mean that future recruitment monitoring could measure the contribution of the spawning periods to the overall recruitment. Further, if the times of spawning are shown to differ along the coast, the relative contribution by spawning areas to a recruitment site could be monitored for annual variation.

Marked changes in behaviour with respect to fish size, from dhufish occurring as solitary individuals or in small groups of no more than five when less than 120 mm in TL to schooling in groups of 30-100 individuals when larger, were recorded during the study. The average size of fish occurring in schools was significantly larger than that of individuals in most surveys. Further evidence for the change from solitary to schooling behaviour was collected on two separate occasions when the numbers of solitary individuals declined and there was a higher number of smaller individuals in the school. It was also noted that a school of juvenile dhufish formed in an area where there had been a number of solitary individuals previously. This behavioural change has been recorded in other similarly long lived reef dwelling species which undergo a habitat shift such as the Nassau grouper (*Epinephelus striatus*). The Nassau grouper has been recorded to occur as solitary individual associated with coral fronds until reaching 160 mm in TL when it shifts to nearby patch reef and can occur in groups of up to 25 individuals (Eggleston 1995).

The agonistic behaviour observed in the smaller solitary juvenile dhufish may explain their sparse distribution and low abundance. These smaller juveniles were closely associated with some form of refuge and regularly took shelter when approached but on a number of occasions if another juvenile dhufish approached the resident would display agonistic behaviour towards the intruding individual to retain access to the refuge. Such behaviour may suggest that the refuge forms a home base upon which they rely and cannot afford to lose or share. It could also suggest that these refuges are in short supply as they need to be guarded so the supply of such refuges could potentially be the limiting factor in dhufish recruitment. A similar situation has been observed for red snapper (*Lutjanus camechanus*) within the Gulf of Mexico where debris and small artificial habitats are regularly occupied by small recruits (Workman *et al.* 2002).

The artificial habitats in areas of sparse seagrass and sand are likely acting as attraction devices, which thereby allowed juvenile recruitment strength to be monitored. The shape and formation of the V-pipes resulted in substantial accumulation of macroalgal wracks which presumably contains a valuable food source for the juvenile dhufish, along with the refuge provided by the hollow pipes. The situation of the main lease in the deeper basin of Flinders Bay may also be important. This area has a propensity for the retention of macroalgal wrack after high swell events. The shape of the bay with the prevailing Leeuwin current creates an eddy which acts to trap/retain the wrack at the main lease sites located in the deepest part of Flinders Bay.

The main lease site and regular sDOV survey technique utilised appear suitable to monitor annual dhufish recruitment, although the validation of this recruitment index in the age structured data would determine its usefulness. Unfortunately due to the full recruitment of the fish to the fishery at 9-10 years of age this validation of the index will take at least 10-15 years.

The success of the “V-pipes” section of the lease at Augusta to consistently hold a school of juvenile dhufish may indicate it is a suitable design to be used for future Artificial Recruitment Monitoring Sites (ARMS) to monitor annual recruitment. Such sites could be established along the WA coast and monitored annually by diver sDOV surveys to enhance the annual recruitment monitoring gathered from the Augusta site. This would develop a robust index of annual recruitment strength which if correlated with the adult age structure in the area could be

used to aid the assessment of the dhufish stocks by and predicting year class strength.

The data recorded at the Augusta site indicates the artificial low profile substrate in predominantly sandy areas is attracting juvenile dhufish from the surrounding area at between 80-130mm in size and they utilise this habitat type until they are 220-280mm in length. This use of a particular habitat type for a short period of their life correlates with the findings of previous studies by Hesp *et al.* (2003) who found three different habitat types are utilised in their early life history.

The high abundance of juvenile dhufish at the Augusta lease on the artificial low profile hard substrate in predominantly sandy areas may indicate there is a shortage of this habitat type in the area. If so the addition of more such artificial habitat may serve to enhance localised recruitment of dhufish. There may be other regions of the WA coast where there is a shortage of low profile reef in predominantly sandy areas and so creation of ARMS along the coast should be assessed for their potential to enhance the localised recruitment of dhufish and similar species. An appropriately designed long term Before After Control Impacted (BACI) study of the dhufish abundance on surrounding areas of natural reef in the vicinity of at ARMS and control sites could potentially if this is the case.

The study has revealed data for juvenile dhufish in the 80 – 240 mm TL size range but there still remain questions on the 20-60 mm size range of new recruits. Information from the current study indicates juveniles of this size are likely to occur as solitary individuals or small groups and be strongly refuge associated in sand inundated reef or seagrass areas, and thus difficult to detect. The images taken of very small juvenile dhufish taken immediately after the artificial habitats were first deployed at the main lease indicated smaller juveniles were present at the main lease site during March to June 2011 but these very small juveniles were not recorded in the sDOV surveys of March to June 2012, after the artificial reefs had become more established. It may be possible that the larger 1yo+ juvenile dhufish were restricting the use of the artificial habitats by the 0+ juveniles and may have been predated upon them, together with other predators such as Rankin cod and breaksea cod. A fine net research beam trawl survey of the surrounding area may reveal their occurrence on such habitat areas and their likely occurrence in low densities.

The sDOV survey technique proved very effective for monitoring the abundance of juvenile dhufish at the sites and also estimating their lengths. The juveniles did not exhibit diver avoidance behaviour, could be readily approached to within 1-3 m allowing footage for accurate length estimates. They also remained in their distinct sections of the lease so preventing double counting. The length estimates for the juveniles and the resulting length frequency data for each survey could be used to assigned abundances to the year classes and also calculate their growth over the course of the study. The video footage and processing software meant the accurate estimation of total abundance and tracking of each juvenile as it moved within the school was possible, which assisted in counting and measuring. The multiple measurements for each individual allowed the length estimate with highest precision to be used.

The regular sDOV surveys documented the increase in the number and abundance of fish species at the main lease site over the course of the year. As with many artificial reef surveys there was an initial increase in the diversity of species on the sites with the addition of artificial structure. However, the diversity quickly stabilised and although over 48 different species were recorded at the main lease site only six species were recorded on every sDOV survey and this included juvenile dhufish. Of these other five species, blue-spotted goatfish and silverbelly are generally associated with sandy habitats while western king wrasse, black spotted wrasse, and snakeskin wrasse utilise a range of habitats including marginal sand inundated reef and low profile reef in sandy habitats. The significance of juvenile dhufish being associated with these

species reinforces that the critical natural nursery habitat of juvenile dhufish is predominantly sandy areas with some form of refuge.

The study has potentially documented a range extension for Rankin cod which are a tropical species that usually occur from the Abrolhos north and are occasionally observed off the Perth metropolitan coast but not previously reported at this southern latitude of Augusta. In recent years after the 2010/11 marine heatwave, this species has, however, been reported as far south as Geographe Bay (www.redmap.org.au). The individual recorded on numerous occasions at the Augusta site was evidently growing and surviving at these southern latitudes over the period from February to October 2012. Additional information on growth of individual breaksea cod and Rankin cod indicate that repeated sDOV surveys would be useful for monitoring biological characteristics such as growth, range expansion and recruitment of a range of species. The initial size of the breaksea cod was that of a three year old and the final size was at that of a five year old, as estimated by Moore *et al.* (2007). Thus, this individual appears to be growing at a rate of twice that estimated by this previous study, which could be due to the high food availability with little competition at this recently created artificial habitat.

3.2 Deployment of artificial habitats in Metropolitan waters

3.2.1 Introduction

This small pilot study was conducted to investigate the feasibility of using small artificial habitats for monitoring juvenile dhufish (TL<150 mm) abundance in the Perth metropolitan area with the view to monitoring annual juvenile recruitment. The first phase was to deploy small artificial habitats at several locations in waters near Perth where juvenile dhufish are known to occur and also into diver-able depths to establish if this technique is likely to succeed. The sites chosen were all areas of at least 200 x 200 m of predominantly sand habitats in the vicinity of low profile reefs and spread through a range of depths extending from 18 m to 32 m. A mixture of towed video, diver sDOV and fish trap sampling were employed to survey the sites before and after the deployment of the artificial habitats.

The ability to monitor dhufish recruitment through the deployment of small artificial habitats could lead to the establishment of a series of recruitment monitoring sites (ARMS) along the WA coast to monitor annual dhufish recruitment. Such an index of recruitment would require a long-term dataset for validation against the age structure but if successful would be an invaluable assessment tool for management advice of future stock levels.

3.2.2 Methods

Three “deep” sites were chosen for the deployment of artificial habitats within an area in waters of Perth that has sometimes commercially trawled and where juvenile dhufish have previously been found by Hesp *et al.* (2003) and Lewis *et al.* (2012). The sites were at depths of 29, 30 and 32 m and were spaced at least 800 m apart (Figure 5.1). The exact locations of the sites were, in part, influenced by the need to avoid the positions in areas of outer ship anchorages used by the Port of Fremantle. The additional sites in shallower depths of 20 and 24 m (and closer to shore) were initially chosen based on aerial photography and lidar (light detection and ranging) mapping, which indicated that these areas were sand habitats close to prominent reefs, and thus potentially habitats of juvenile dhufish. The exact positioning was also required to avoid an exclusion zone containing communication cables.

Initially, in December 2011, proposed monitoring locations were surveyed using a towed underwater video system to determine the precise co-ordinates of areas with suitable and predominantly sandy habitats (Table 5.1). When processing the video surveys, the benthic habitat types were mapped by transcribing the coordinates off the video for each habitat type and mapping these in ArcGIS. As video were processed the fish species observed in the area were recorded before deploying the artificial habitats.

The artificial habitats in the trawl area were surveyed seven days after deployment in January 2012 using the towed live feed stereo video system. All sites except the western trawl area site were re-surveyed 50-60 days after deployment by divers with sDOV on the 23rd of February 2012. In addition, the trawl area sites were sampled 69 days after deployment on the 6th of March 2012 using large baited fish traps.

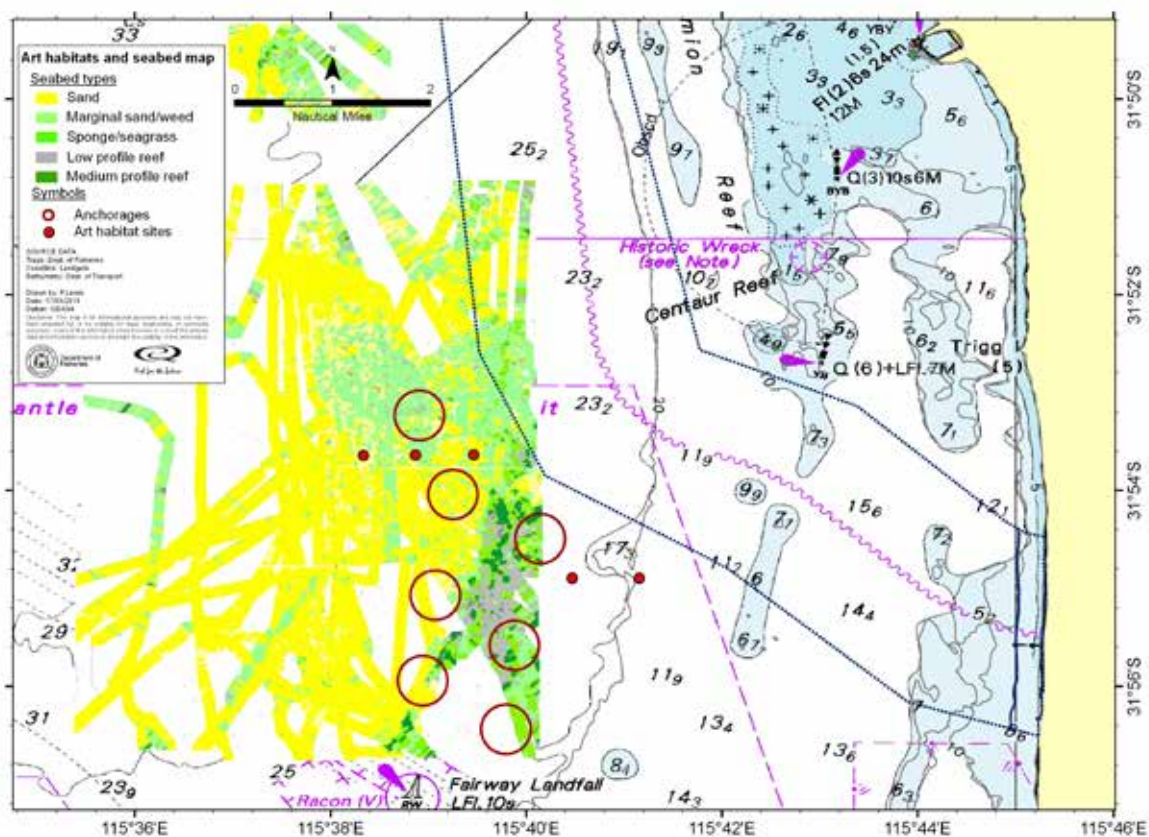


Table 5.1 Details of depth, habitat and dates for artificial habitat deployment and surveys of Perth metropolitan artificial habitat study sites.

Site	Depth	Habitat	Pre-survey	Deployment	Towed survey	sDOV Survey	Trap survey
Trawl west	32	Sand	19/12/11	21/12/11	28/12/11		6/3/12
Trawl central	30	Sand/ inundated reef	19/12/11	21/12/11	28/12/11	23/2/12	6/3/12
Trawl east	29	Sand	19/12/11	21/12/11	28/12/11	23/2/12	6/3/12
Inshore west	24	Sand	28/12/11	11/1/12		23/2/12	
Inshore east	18	Sand	19/12/11	28/12/11		23/2/12	

3.2.3 Results

Pre-deployment

All of the sites surveyed were areas of predominantly open flat sand except for the central trawl site that had some sparse weed and sponge, indicating small areas of sand inundated reef (Figure 5.3). The only fish observed at the sites on the towed video were a small number of whiting (*Sillago* sp.) at the east and west trawl sites, and a school of approximately 20 small trevally (*Pseudocaranx* sp.) at the west trawl site (it was not possible to determine exact numbers due to poor visibility and video resolution).

The previous bottom type discrimination surveys of the trawl area indicated the three sites were predominantly sand habitat with some marginal reef areas that were partially inundated by sand (Figure 5.2). No such surveys were available for the inshore sites. The underwater video surveys confirmed the patches of sand inundated reef in the vicinity of the central trawl site and a reef edge only 70 m from the 24 m site (Figure 5.2). On this reef edge, an adult dhufish was recorded along with reef associated species such as small trevally, western king wrasse, and West Australian pullers (*Chromis westaustralis*).

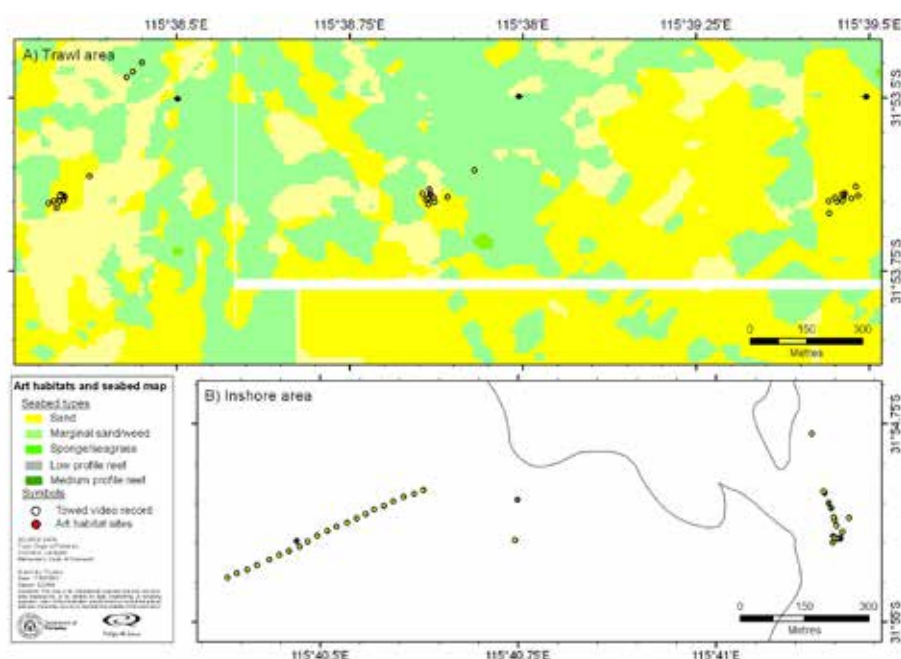


Figure 5.2 Map of benthic habitat types identified in vicinity of ARM sites in A) the trawl area and B) the inshore area by Sea Scan discrimination and towed video observations.

Post deployment

The towed stereo video survey of the trawl area sites a week after the artificial habitats were deployed indicated fish species including western king wrasse (*Coris auricularis*) and western butterflyfish (*Chaetodon assarius*) were utilising the habitats on each of the three sites. A small fish was observed inside the hollow of one block on the west trawl site but could not be identified from the towed video footage.

On the diver sDOV survey, two juvenile dhufish were recorded at the central trawl area site (Figure 5.2) but none at the other three sites surveyed (trawl east and inshore sites). These two individuals were estimated by Eventmeasure to be 142 and 151 mm TL. In the trapping survey, two juvenile dhufish were caught at the western trawl area site which measured 133 and 134 mm TL. At this size and the time of year, all of the juveniles were likely to be 1+ year old recruits that had been spawned in 2010/11.

During the diver surveys it was noted that juvenile dhufish were quite aggressive towards one another with a larger individual chasing the smaller one away from the artificial habitat. It was also noted that the smaller juvenile used a nearby patch of sand inundated reef habitat (Figure 5.3).



Figure 5.2 Images of a juvenile dhufish associated with the Perth metropolitan artificial habitats.

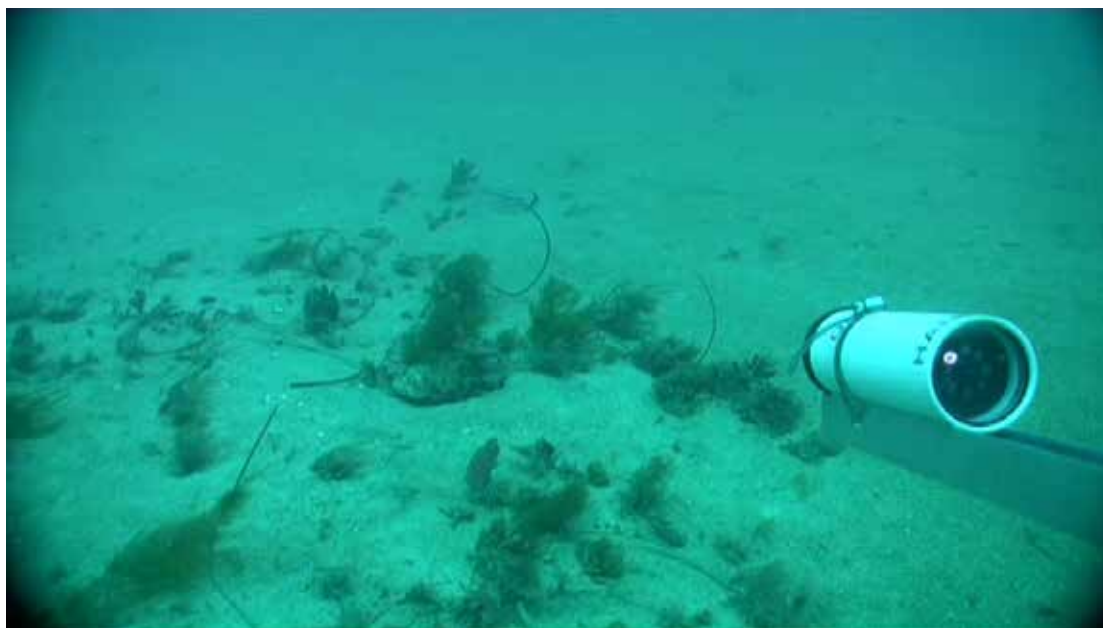


Figure 5.3 Image of a juvenile dhufish associated with a small patch of sand inundated reef habitat in the vicinity of the central trawl site.

The nine other fish species observed at the artificial habitat sites were dominated in abundance by the western king wrasse. Almost all other species were only observed as single individuals (Table 5.2). It was noted on the sDOV surveys that the blocks at the trawl east and inshore sites were coated in a thick layer of green filamentous algae (Figure 5.4) that was not observed on the blocks at the central trawl site.

Table 5.2 Summary of fish species recorded at artificial habitat sites on sDOV survey.

Species	Trawl central	Trawl east	Inshore west	Inshore east
<i>Glaucosoma hebraicum</i>	2	-	-	-
<i>Austrolabrus maculatus</i>				1
<i>Chaetodon assarius</i>	2	1		
<i>Chelmonops curiosus</i>		1	1	
<i>Chromis westaustralis</i>		1		
<i>Coris auricularis</i>	37	52	50	10
<i>Eupetrichthys angustipes</i>	1			1
<i>Parapercis haackei</i>	1			
<i>Parupeneus chrysopleuron</i>	1			
<i>Trachurus novaezelandiae</i>	1			

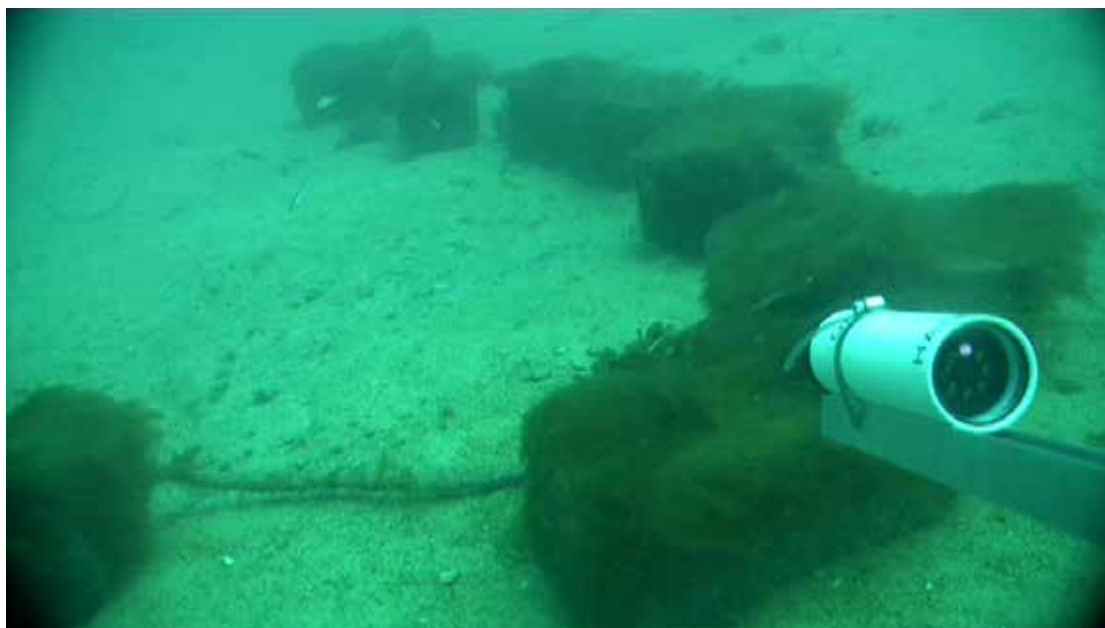


Figure 5.4 Image of artificial habitat at the inshore site covered in green filamentous algae.

3.2.4 Discussion

The small scale trial using small artificial habitats in the Perth metropolitan area was successful in recording juvenile dhufish at two of the five sites. The results indicate the establishment of small artificial habitats in areas of suitable marginal sand inundated reef habitat appears to be an effective method for locating and concentrating juveniles to estimate abundance. The effectiveness of the small artificial habitats may indicate that there is a limitation of this habitat type for dhufish recruitment and deployment of larger scale low profile artificial habitats may enhance annual juvenile dhufish recruitment. The sites were effectively sampled by two techniques (Trap and sDOV), with the sDOV providing more information on the total abundance, associated species and behaviour, but this diver operated sampling is limited by depth. However, the effort required to use this technique to monitoring recruitment was minimal compared to that expended on other larger scale surveys which recorded lower numbers of juveniles in the same area (Section 6.0). The trial deployments and monitoring of small artificial habitats showed promise for the future monitoring of annual juvenile dhufish recruitment. However, the lack of juveniles at the inshore sites may indicate the need to establish sites in a range of habitats, depths and areas along the coast to adequately monitor annual recruitment strength.

The result of two juvenile dhufish on each of the two deeper sites within three months of the artificial habitats being deployed suggests the use of artificial habitats has potential for monitoring of annual dhufish recruitment. The masonry blocks used appear to be suitable as an artificial habitat for small juvenile dhufish, less than 150 mm TL. The expansion of the artificial habitats to more than a few masonry blocks may increase the number of juveniles able to reside on each site and so enhance the viability of the method to be a measure of annual recruitment. The appearance of juveniles within such a short time frame, with few benthic invertebrates on the artificial hard substrate to provide an additional food resource, indicates they are predominantly using the blocks as a refuge.

The juveniles were recorded by two different sampling techniques although the sDOV provides a better overall assessment of the site than fish traps but is restricted in the depths that can be

sampled. The lack of juveniles on the inshore sites may indicate they do not occur in shallower depths, but the occurrence of the smothering green filamentous algae over the blocks may have affected the suitability of the blocks as refuge for juvenile dhufish. The project has sampled the deployed artificial habitats by a number of methods including traps and towed video but sDOV appears best as have more control and can get better estimates of numbers and sizes at a site. Although the trial has demonstrated that it is not always possible to find suitable areas in diverable depths for monitoring by this method.

Prior to deployment of the artificial habitats at each site the surveys indicated only sand associated species such as whiting and small trevally in the vicinity. The sDOV surveys provided the most comprehensive assessment of the sites with the trap and towed video giving few details on the species present at the sites. The highest diversity of seven species was recorded at the central trawl site where the juvenile dhufish were also recorded. Of note was the snakeskin wrasse (*E. angustipes*) which has occurred in previous surveys and at the Augusta site and appears to be associated with the same habitat as juvenile dhufish.

The agonistic and seemingly territorial behaviour observed in the juvenile dhufish on the Perth metropolitan artificial habitat trial has also been recorded at the Augusta lease site (Section 3.1.3). This behaviour may explain why juvenile dhufish are solitary and widely distributed although as this behaviour changes as they grow to become schooling. It may also indicate that only a small number of juveniles will occupy such a small area of artificial habitat as that used in the current study due to this behaviour. Such agonistic behaviour may limit the number of recruits a small artificial habitat site can hold requiring the establishment of larger structures similar to the V-pipes formation at the Augusta abalone lease which could increase the available habitat and hence numbers of juveniles at each site and provide a more robust annual index of recruitment.

The observed retreat of the smaller juvenile dhufish to a patch of sand inundated reef as a refuge may confirm the type of natural habitat that juvenile dhufish are typically associated with in the area. This further confirms the results of the previous survey (Lewis *et al.* 2012) of juvenile dhufish natural habitat being patches of sand inundated reef in predominantly sandy habitat areas.

The effort required to survey and deploy a few small artificial habitats in the Perth metropolitan trawl area and successfully record a small number of juvenile dhufish (n=4) was minimal compared to the effort required to conduct a trawl, trap and video survey of the same area. The effort required to survey, deploy and resurvey the 5 sites was a total of 3 small boat days with 3 personnel resulting in 4 juveniles recorded. The time required on the larger trawl, trap and BRUVs survey requiring a larger research boat to survey the nearby area was 6 days with 6 personnel resulting in only 1 juvenile dhufish (Section 6).

The appearance of a few juvenile dhufish on such a small trial of artificial habitat may indicate that there is a paucity of the low profile reef in areas of predominantly sandy habitat that is critical to juvenile dhufish annual recruitment. This form of habitat appears to act as a refuge that juvenile dhufish require in the first few years. The deployment of artificial habitats in a long-term Before After Control Impacted (BACI) designed study to determine if such sites have an influence on the abundance of dhufish on the surrounding natural reef areas. Such a long term trial could be conducted where monitoring of surrounding natural reef areas in similar locations where low profile artificial habitats are added and another where they are not. As the movement of recruited juveniles and adult dhufish is thought to be limited (Fairclough *et al.* 2013) any increase in the local recruitment of dhufish by ARMS to the surrounding reefs should be measurable.

3.3 Annual abundance of juvenile dhufish in Perth metropolitan trawl area

3.3.1 Introduction

Previous research surveys conducted in the Perth metropolitan trawl area have detected juvenile dhufish in low numbers by three different methods (Hesp *et al.* 2003, and Lewis *et al.* 2012). These methods were paired otter trawls, small opera-house fish traps and BRUVs. The overall objective in 2012 was to repeat some of the successful sampling methods from 2011 in the Perth metropolitan scallop trawl area and compare results, particularly catch rates with the previous surveys. The ability to conduct such an annual survey which may identify annual fluctuations in the abundance of juvenile dhufish by these methods within the Perth metropolitan trawl area would allow prediction of future recruitment variation into the fishery.

Sampling was undertaken in March 2012 and the catch rate compared to the previous surveys (Lewis *et al.* 2012) and historical trawl surveys in 2003. The same successful methods of trawling, baited remote underwater video (BRUV) and overnight fish trap sets were employed in the Perth metropolitan scallop trawl area. Sampling in 2012 was to a lower degree than in previous surveys for all methods but did obtain a few juvenile dhufish by three methods allowing catch rates to be compared with previous surveys.

3.3.2 Methods

Sampling was conducted in the Perth metropolitan trawl area using a variety of methods from the 5th-9th of March 2012. To sample the fish community in the area various types of baited fish traps such as operahouse, large commercial traps covered in fine mesh and crab traps (described in Lewis *et al.* 2012) (n=34) were set in the area overnight, BRUVs (n=5) were set during the day and try-net trawls (n=22) over a distance of 1 nm were conducted both day and night. The catch rates of juvenile dhufish were calculated and the results compared to those obtained in previous surveys conducted in September 2010, February 2011 and a trawl survey in April 2003.

To calculate the area sampled by each trawl, the sampling width of each net type was first determined by multiplying the length of the head rope by the estimated lateral spread. The lateral spread was assumed to be 75% of the headrope length as the effective lateral spread is generally 60-85% of the length (Kangas *et al.* 2006). The 2003, 2010 and 2011 surveys all used paired otter rig trawl nets of 7 fathom head rope length. The 2012 survey used a single try-net with a five fathom head rope length. Thus, the effective sampling widths for each twin otter rig and try-net trawl were approximately 19 m and 6.75 m, respectively. The total area sampled by each trawl was calculated by multiplying the distance of the trawl, usually 1 nm, by the sampling width. From these, the total area sampled in each survey period was calculated and this was divided by the number of juvenile dhufish collected to give an estimate of the area sampled for each individual.

3.3.3 Results

Four juvenile dhufish were recorded in the 2012 field sampling by all three of methods. Although two of the juveniles were caught in a fine mesh covered large fish trap which was deployed in an overnight set on the artificial habitat sites (Section 3.2). The try-net trawling and BRUVs each sampled one individual juvenile dhufish.

Fish trap

The two juvenile dhufish sampled by the large meshed fish trap were caught at the west trawl

artificial recruitment monitoring site. These two juveniles were 133 and 134 mm TL and were utilised in the study of juvenile dhufish diet (Section 4.0). A total of 24 fish species were sampled by the fish traps (Appendix 1).

BRUV

A single juvenile dhufish was observed on the single camera BRUV set in the trawl area on 14th Oct 2011. As this was not a stereo BRUV, the length could not be estimated accurately. As this fish was observed in front of the bait basket, the size could only be estimated from the width of the bait pole at approximately the same distance. The length was estimated to be six times the width of the bait pole, which is 25 mm in diameter giving an estimated TL of 150 mm. Only 23 different species of fish were recorded on these BRUV sets (Appendix 1). Most of these sets were on sand or marginal sand inundated reef habitat.

Trawl

The single juvenile dhufish sampled by the try-net trawl sampling in 2012 was 122 mm TL. The sampling recorded 55 other fish species (Appendix 1). The survey also sampled a large number of saucer scallops (*Amusium balloti*) and recorded size frequency data for over 500 of these to be used in the assessment of the scallop trawl fishery the area.

Although the try-net used in 2012 differed to the paired otter net trawls used in the previous surveys the number of trawls conducted was higher and the calculated area sampled was similar (Table 6.1). Thus, at the low catches of juvenile dhufish in each year the rate of 1 juvenile dhufish for every 289,702 – 432,440 m² sampled by trawling.

Table 6.2 Summary of research trawl sampling and catch of juvenile dhufish by year in the Perth metropolitan trawl area.

Year	Month	Type	Width (m)	No. of trawls	Area sampled (m ²)	No. of juveniles
2003	Apr	Twin otter	19	13	432,440	1
2010	Sept	Twin otter	19	19	579,405	2
2011	Feb	Twin otter	19	11	357,200	1
2012	Mar	Trynet	6.75	22	399,600	1

3.3.4 Discussion

The sampling in 2011/12 of the Perth metropolitan trawl area by a range of techniques was successful in obtaining a low number of juvenile dhufish (n=4, TL<150 mm). The juveniles were recorded by three different sampling techniques trawl, BRUV and large fish trap. The low numbers collected may indicate the low abundance of juvenile dhufish in the Perth metropolitan trawl area during 2011/12 and that such a survey is not sufficient to detect recruitment variation.

Juvenile dhufish were collected by similar methods to the previous years surveys with low numbers recorded by fish traps (n=2), BRUVs (n=1) and trawling (n=1). The large meshed fish traps were successful in 2012 whereas the smaller opera-house fish traps were in 2010/2011 (Lewis *et al.* 2012). This may indicate it is the location and not necessarily the type of trap, other than having fine mesh to retain small dhufish, which is important. The small opera house traps were the previous only successful trap type but the effort was far greater than the other trap types and they effectively sampled more sites in 2010/11 than any other type of fish trap. The success of the large fish traps in 2012 was enhanced by being set on the artificial habitat

sites which were established a few months earlier, again indicating it is the location and not necessarily the trap type that is important.

The observation of a single juvenile dhufish on a BRUV in 2012 was another example of a chance event of BRUVs recording juvenile dhufish. The BRUV was only deployed in the Perth metropolitan trawl area to gather footage of it going in the water for a television production. The visibility was very poor at less than 1 m and the footage was only processed out of curiosity. As this was only a single camera and not a stereo BRUV so the size of the juvenile could not be accurately measured but based on its location and width of the bait pole it was estimated to be 160 mm in length.

The comparison of trawl sampling effort with previous surveys showed that although the try-net used in the current study had a smaller sampling area than the paired otter net trawls used previously the catch rate of juvenile dhufish by area sampled was similar between years. The abundance of juveniles in the area has been low at one for every 3-400, 000 square meters of seabed sampled by trawling for all surveys since 2003.

3.4 Additional site and artificial habitat surveys

3.4.1 Introduction

The anecdotal reports collated during the first field component of the project indicated several areas of interest requiring further investigation as potential juvenile dhufish nursery areas (Lewis *et al.* 2012). These areas included sites off Bunbury, Castle Rock, Dunsborough, and south of Rottnest Island along with the investigation of the established artificial habitats of the *HMAS Swan* and Quindalup tyre artificial reef.

The *HMAS Swan* (Length: 112 m, Height: 21 m) was sunk as an artificial dive reef on 14th December, 1997 and has a 200 m fishing exclusion zone surrounding it. A long term study has documented the fish community at the site and at nearby reference sites before it was sunk and at regular intervals after (Morrison 2003). The species observed on the wreck during these surveys included dhufish which occurred seasonally although no sizes were estimated.

The Quindalup tyre artificial reef was established in the late 1980s and covers a wide area of sand and seagrass beds where tyres in various configurations such as tetrahedrons, tubes and rows were deployed. There is little documented evidence on the fish species occurring on the tyre reef but the depth of 22 m and surrounding habitat types deemed it potentially suitable for juvenile dhufish.

3.4.2 Methods

The majority of the sites surveyed for juvenile dhufish in the western Geopraphe Bay region (Figure 7.1) were surveyed from the 16-20 January 2012 with the additional sites off Bunbury and south of Rottnest Island surveyed on subsequent trips. The general characteristics of each site are given in Table 7.1. Initially each site was surveyed by towed video to determine the benthic habitat types, areas of critical habitat and the location of features, for details of the towed video system and methods (see Section 2.0). The other methods employed to survey the fish species varied between sites and included sDOV, small fish traps and BRUVs. The methods used at each site are given in Table 7.1. A general description of each method is given in Section 2.0.

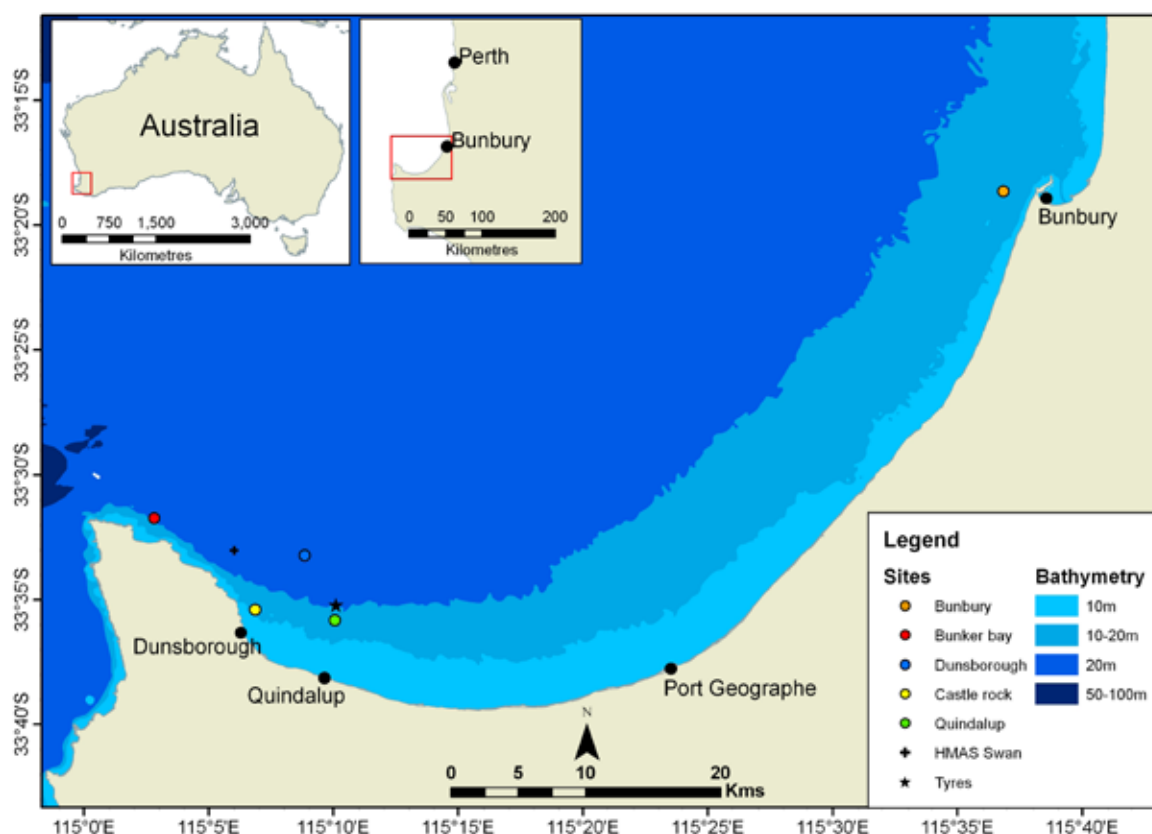


Figure 7.1 Location of sites in the southwest of WA surveyed for juvenile dhufish.

Table 7.1 Sites surveyed with depth, main habitat types and methods used to survey.

Site	Depth (m)	Habitat types	Method/s			
			Tow video	sDOV	BRUV	Trap
Bunbury	17	Sand/seagrass/ low profile reef	Y		3	
Castle rock	8	Sand/seagrass/ small outcrops	Y	Y		
Dunsborough	27	Sand/seagrass/ low profile reef	Y		3	
Quindalup	22	Seagrass/sand	Y		5	6
South Rottneest	32	Sand/seagrass/ low profile reef	Y		2	12
<i>HMAS Swan</i>	31	Sand/sand inundated reef		Y		
Tyre reef	22	Seagrass/sand	Y	Y	2	4

3.4.3 Results

No juvenile dhufish were observed at any of the seven additional sites investigated even with effort by a number of different methods at most sites. The surveys did record a total of 86 different species at these sites (Appendix 1) and mapped the benthic habitat types in these areas. Details of the benthic habitat types and diversity of fish species found at each of the sites are given below along with a comparison of methods at the tyre reef and Quindalup sites.

Bunbury

The sampling at the Bunbury site was primarily focused on mapping the benthic habitat for the installation of a future artificial reef. The benthic habitat at the site was composed of a mixture of sand, seagrass (predominantly *Amphibolis* sp.) and an area of low profile reef (Figure 7.2). The wide ridges of sand are clearly evident in this sidescan sonar survey of the area. These habitats were confirmed with towed video and diver observations.

The towed video survey was able to detect 17 different species of fish in the area (Appendix 2). The majority were observed on the section of seagrass and low profile reef located in the northwest of the site and only an eagle ray was observed on the sand.

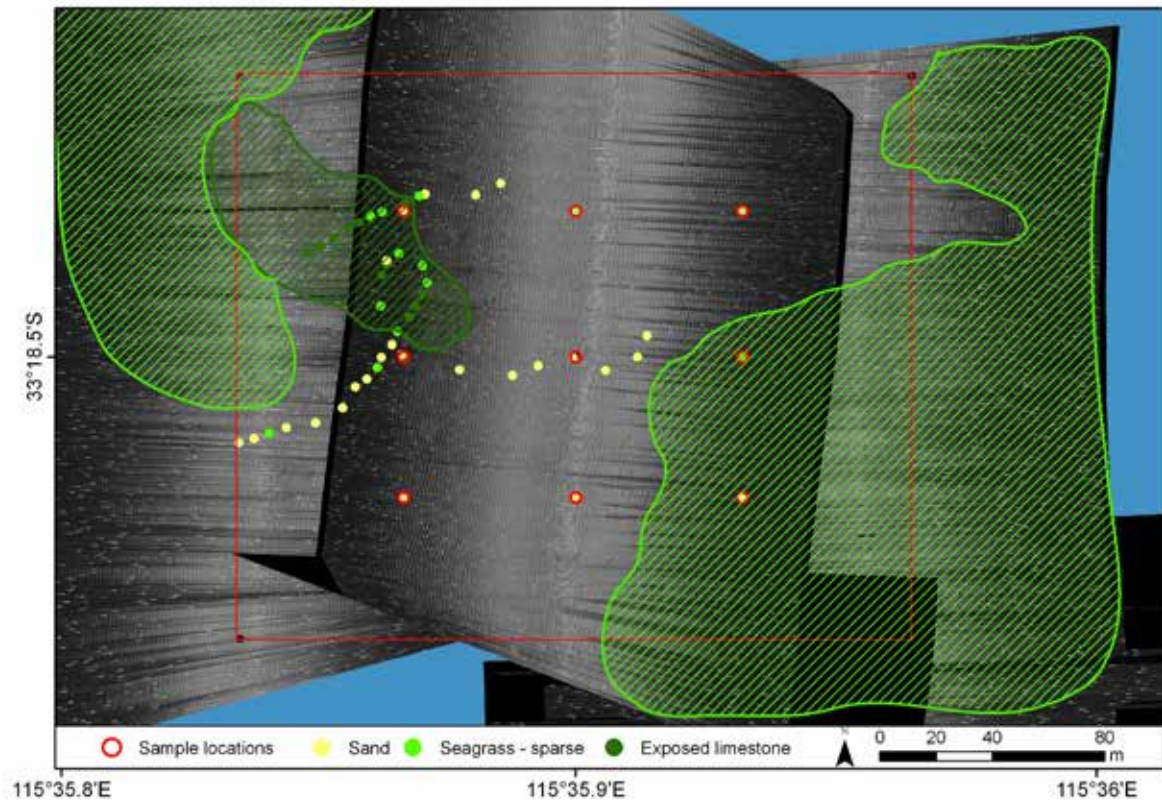


Figure 7.2 Sidescan sonar map of Bunbury site indicating towed video benthic habitat types, sampling locations and general areas of seagrass (light green) and low profile reef (dark green).

Castle Rock

The majority of the benthic habitat in the area surveyed was mixed *Posidonia* and *Amphibolis* sp. seagrass beds with some patches of bare sand between and a few small emergent limestone outcrops of < 0.5 m in height scattered through the area (Figure 7.3).

The sDOV and towed video surveys recorded only 12 different species of fish at this site (Appendix 2). These included breaksea cod and redlined cardinal fish (*Apogon victoriae*) associated with the limestone outcrops (Figure 7.4).

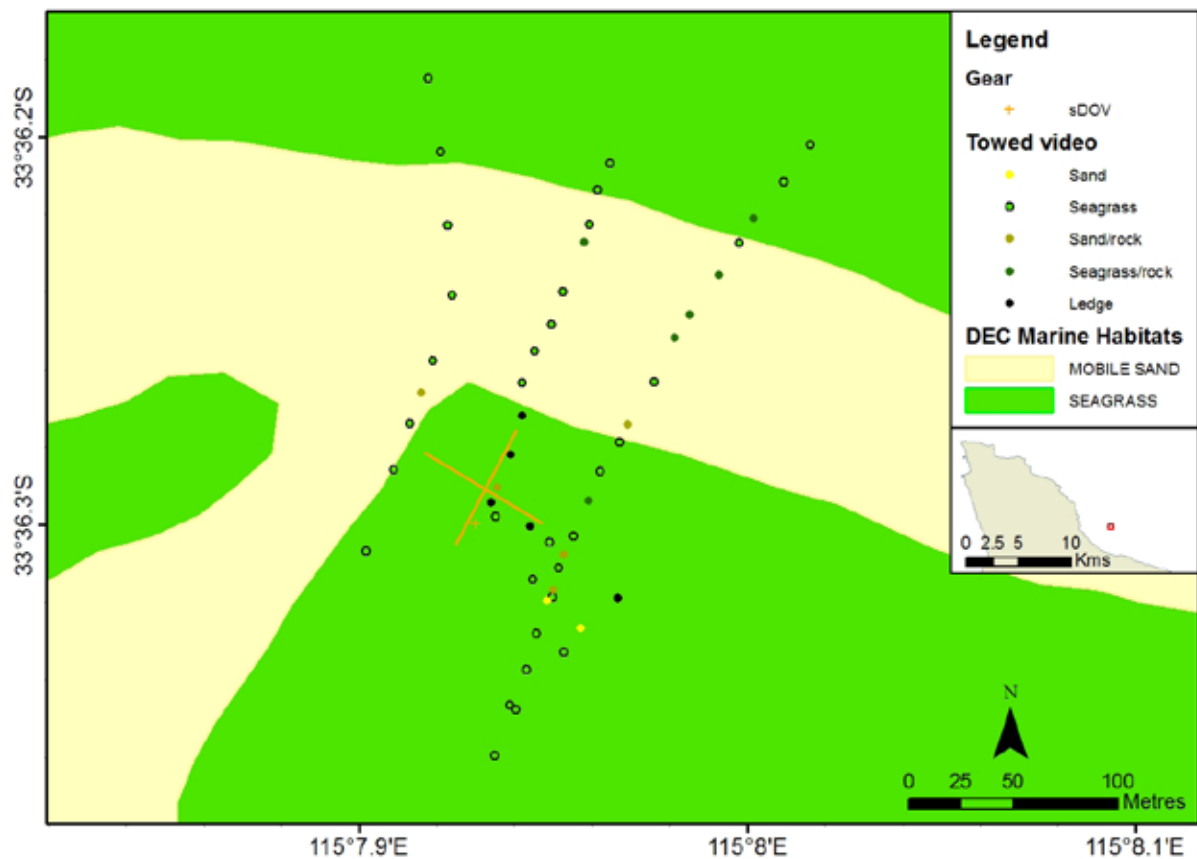


Figure 7.3 Map of the site off Castle rock indicating DEC habitat mapping, towed video benthic habitat type observations and sDOV survey.



Figure 7.4 Image of low relief limestone outcrop surrounded by *Amphibolis* sp. seagrass and patches of sand.

Dunsborough

The sampling at the Dunsborough site was primarily focused on mapping the benthic habitat in the area designated for the installation of a future artificial reef. The site was predominantly bioturbated sand with some shell with some small patches of seagrass (predominantly *Posidonia* sp.) and an area of low profile emergent limestone reef to the northeast (Figure 7.5).

The towed video survey was able to detect 12 different species of fish (Appendix 2). The majority of these were sand associated species such as rays and stingarees that may be feeding on the shell beds in the area.

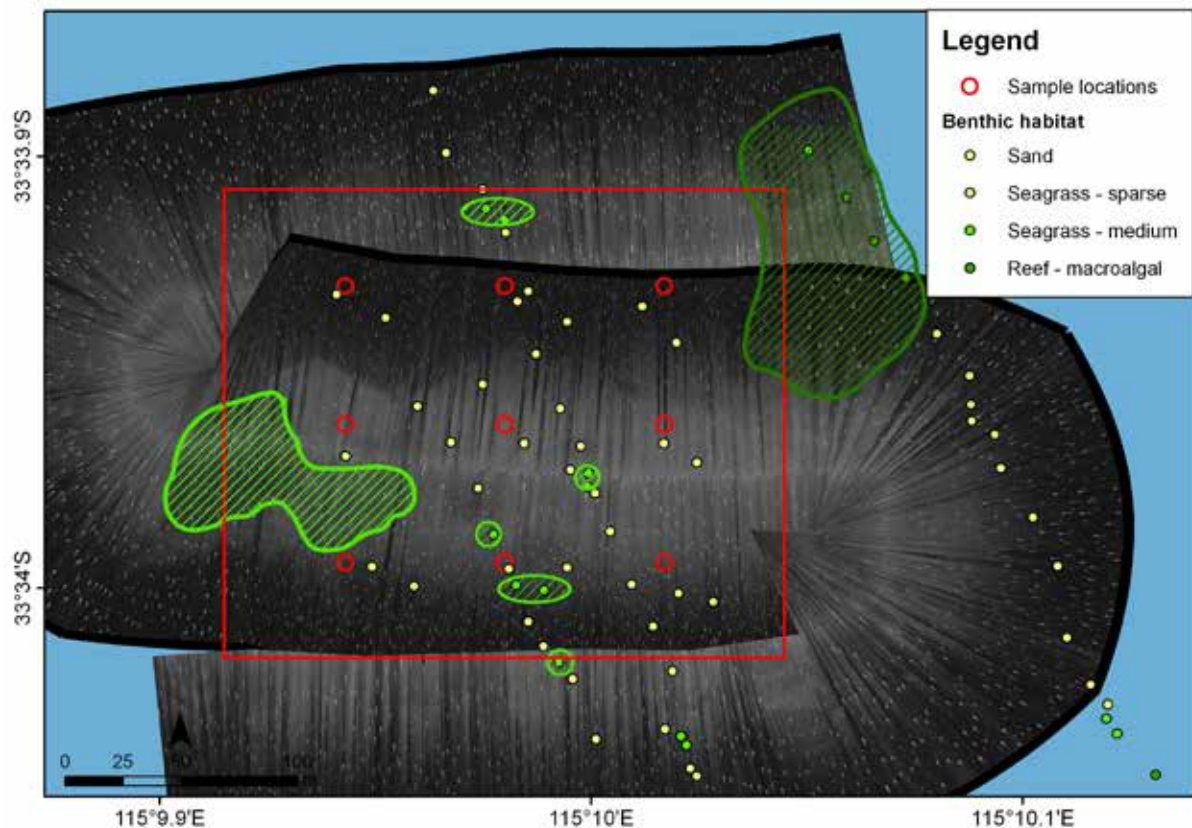


Figure 7.5 Sidescan sonar map of site off Dunsborough indicating towed video benthic habitat types, sampling locations and general areas of seagrass (light green) and low profile reef (dark green).

Quindalup

The towed video survey of the Quindalup site covered a large area of approximately 700 m X 700 m which was of predominantly dense *Posidonia* and *Amphibolis* sp. seagrass beds but also including some patches of bare sand, low profile limestone reef outcrops, and a large *Turbinaria* coral bombie (Figure 7.6).

The towed video, BRUV, and trap surveys of the area recorded 46 different species of fish (Appendix 2) including one small dhufish (TL=320 mm). The small dhufish was recorded by towed stereo video associated with a large isolated *Turbinaria* sp. coral bombie surrounded by *Posidonia* sp. seagrass in the east of the site (Figure 7.7).

By method the most species (n=35) were recorded by the 5 SBRUV sets and of these the set on

the low profile emergent limestone reef to the west recorded the highest diversity of 25 species compared to the 9-13 recorded at on the other sets amongst seagrass. However, the BRUV drop at the *Turbinaria* sp. coral did not record the dhufish or breaksea cod at the eastern site that was observed on the towed video but did record the boarfish (*Paristiopterus gallipavo*). The towed video also proved an effective method by recording 22 different fish species, most of which (n=17) were also recorded by the BRUVs. The traps only recorded six species however four of these were not recorded by the other two video methods.

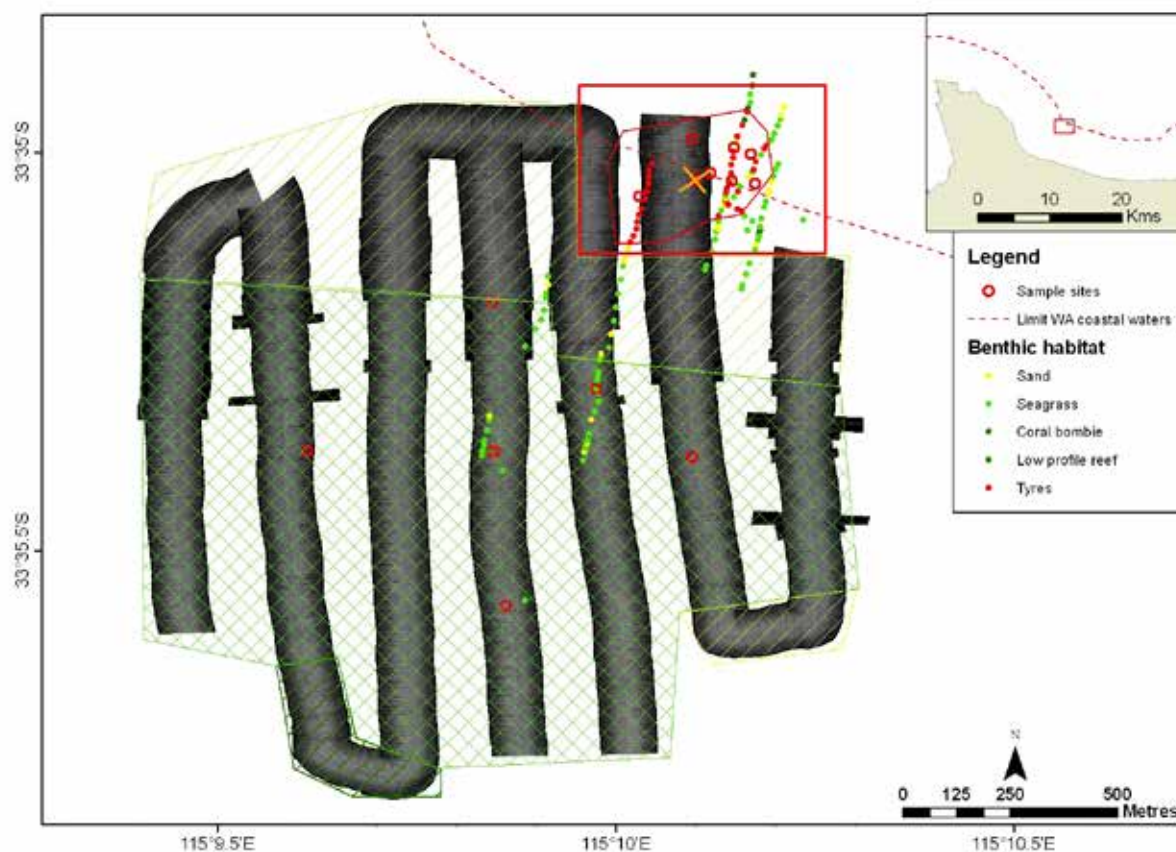


Figure 7.6 Sidescan sonar map of Quindalup site showing locations of gear sets, towed video benthic habitat observations and indicating general areas of patchy seagrass (light green hatching), dense seagrass (light green double hatching), low profile reef (dark green hatching), sand (yellow hatching) and area of Quindalup tyre reef (red).



Figure 7.7 Image of small dhufish (TL=320 mm) recorded in vicinity of large *Turbinaria* sp. coral to the east of the Quindalup site.

South Rottnest

The towed video habitat mapping of three sites within the area indicated a range of habitat types from open sand to sand inundated reef and low profile reef edges in the vicinity of the southern site and predominantly reef and sand inundated reef at the other two sites (Figure 7.8).

The BRUV and fish trap surveys recorded a total of 28 different fish species at these sites (Appendix 2). There were only four species in common to both methods (*Chromis westaustralia*, *Coris auricularis*, *Neotypus obliquus* and *Pseudolabris biserialaris*) of the 19 and 13 species recorded by BRUVs and traps, respectively.

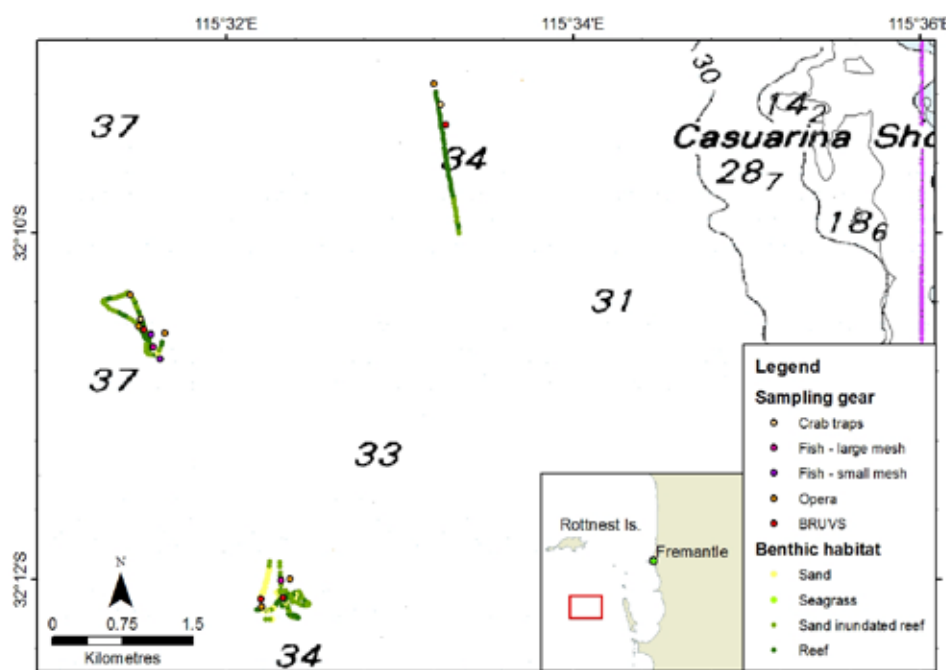


Figure 7.8 Map of bathymetry at towed video sampling sites south of Rottnest Island indicating benthic habitat types.



Figure 7.9 Image from BRUV of sand inundated reef habitat and fish species at south Rottnest area.

HMAS Swan

The sDOV survey of the *HMAS Swan* on the 19th of January 2012 recorded 319 individual fish from 27 different species (Appendix 2) including a single small dhufish with an estimated TL of 325 mm (Figure 7.10).

The benthic habitat surrounding the wreck is a mixture of bioturbated sand, patches of sand inundated reef, rubble and low profile limestone outcrops evident within close proximity (Figures 7.11a and 7.11b).



Figure 7.10 Image of small dhufish (TL = 385 mm) recorded at *HMAS Swan* dive site.

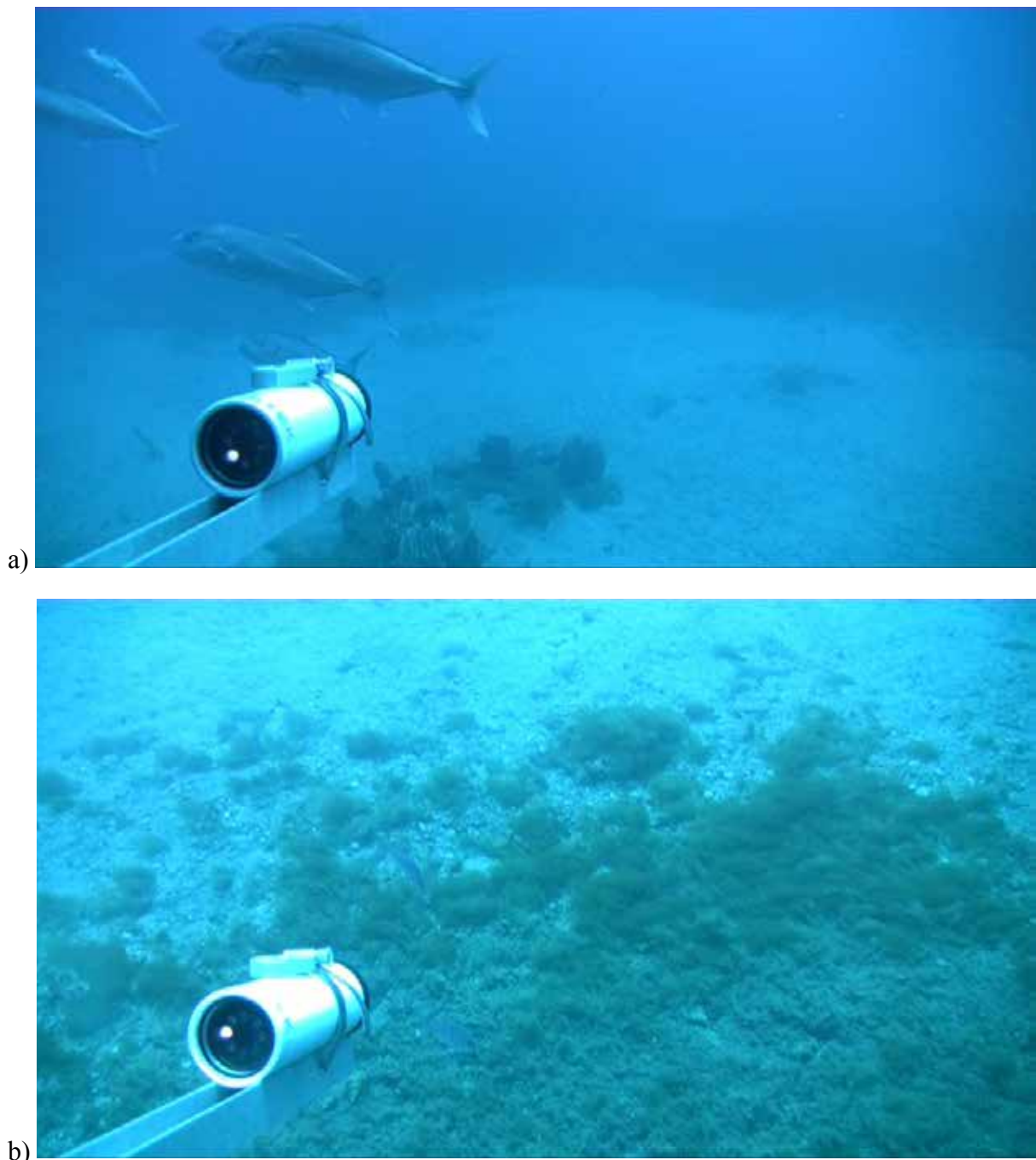


Figure 7.11 Images of benthic habitat surrounding HMAS Swan illustrating a) samsonfish (*Seriola hippos*) with an area of low profile limestone reef with sponges surrounded by bioturbated soft sediment and areas of hard substrate (dark) in background and b) juvenile snapper (*Chrysophrys auratus*) on nearby areas of hard substrate with macroalgae (dark), areas of soft sediment (light) and areas of rubble and shell at transition between.

Quindalup tyre reef

The habitat mapping at the Quindalup tyre reef indicated the tyres were spread through a large area of at least 300 x 300 m (Figure 7.12). The benthic habitat in this area consists of predominantly bioturbated sand with patchy but dense seagrass beds scattered through the south and west (Figure 7.12). The tyres were in a number of formations ranging from scattered

individual tyres laying flat on the seabed to intact tyre pyramids and rows of four or five upright tyres (Figure 7.13).

The BRUV, fish trap, sDOV and towed video surveys recorded 37 different fish species at this site (Appendix 2). Only three recreationally significant species were recorded at the site of which silver trevally (*Pseudocaranx dentex*) was the fourth most abundant species while Samson fish (*Seriola hippos*) and breaksea cod (*Epinephelides armatus*) were in low numbers.

By method the most species (n=25) were recorded by the sDOV survey of the site followed by the two BRUV sets at n=20. In comparison the towed video and traps recorded only very few species at n= 10 and n=3, respectively. Of these species only one by each method was not recorded by the video methods.

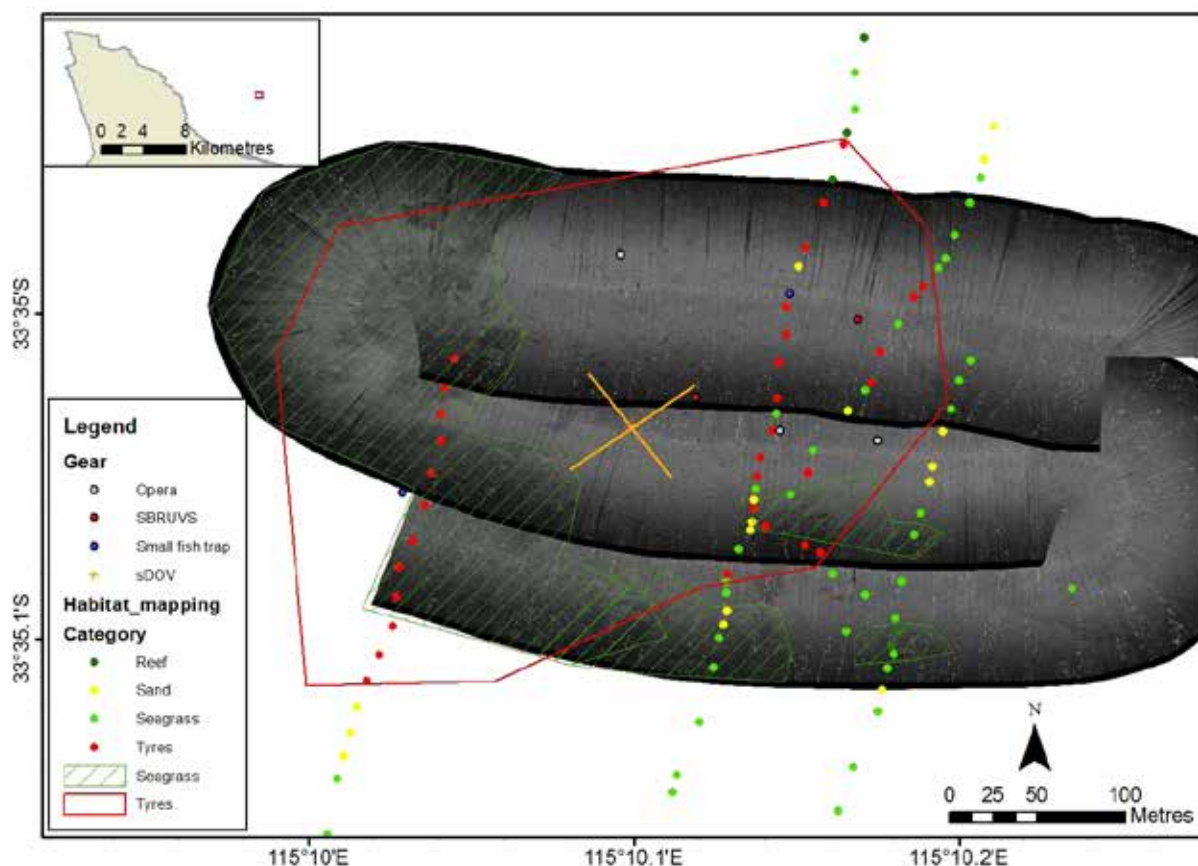


Figure 7.12 Sidescan sonar map of Quindalup tyre artificial reef site showing sampling locations, towed video benthic habitat observations and indicating areas of seagrass (green hatching) and area with tyres (red).

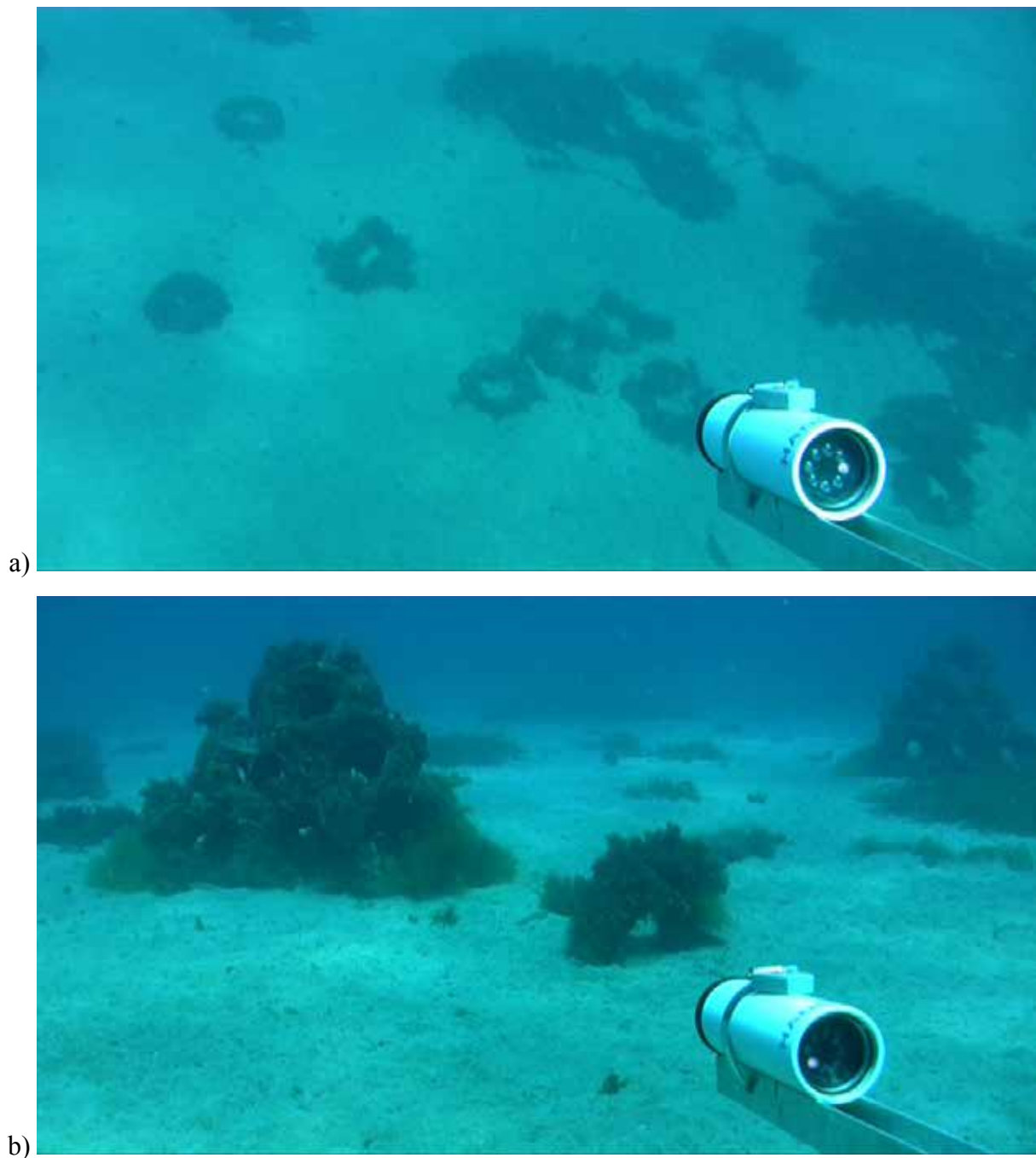


Figure 7.13 Image of a) scattered loose tyres and b) intact tyre pyramids at the Quindalup tyre artificial reef.

3.4.4 Discussion

Despite utilising a range of techniques at seven different sites spread through the southwest of WA no juvenile dhufish were recorded. The techniques employed at these sites have all previously detected juvenile dhufish (Section 6.0), particularly the sDOV at the Augusta lease site (Section 2.0). The surveys recorded a total of 86 different fish species at these sites. Each site investigated had a mixture of habitat types including sand, mixed seagrasses and low profile or sand inundated reef so were potentially suitable as nursery habitat for juvenile dhufish.

Given the success of the sDOV survey methods at the Augusta abalone lease and Perth metropolitan artificial habitat trial it is likely that if juvenile dhufish were present at any of

these sites they would have been detected. Larger dhufish were recorded at two of the sites including the *HMAS Swan* and the area off Quindalup. One notable species in the species list is the snakeskin wrasse (*Eupetrichthys angustipes*) which has been recorded at both the Augusta and Perth metropolitan sites with juvenile dhufish (Sections 3 and 5). This species was recorded at the *HMAS Swan*, artificial tyre reef and Dunsborough sites and may be a useful indicator species of suitable habitat for juvenile dhufish at these sites.

At the two sites (Tyre reef and Quindalup) where a number of methods were conducted the sDOV and BRUV recorded the highest diversity of species while the towed video and traps only recorded a few additional species. The towed video did prove effective in being the only method to record a dhufish at the Quindalup site and is an essential method for habitat mapping and locating areas of habitat diversity. The trapping was not effective and would not be repeated in the future as it proved the least effective and time consuming.

The surveys of the Bunbury and Dunsborough sites served as pre-deployment habitat assessments for the proposed artificial reefs to be deployed in early 2013. These surveys showed the range in habitat types found within a small 200 x 200 m area including sand, seagrass beds and emergent low profile limestone reefs. This range in habitat types also included marginal transition zones of sand inundated and sparse seagrass beds which have been shown to be important for juvenile dhufish. These sites are to be assessed on an ongoing basis after the deployment of the artificial reefs and although there was a lack of juvenile dhufish on these initial surveys, the habitat in the area appears suitable and thus juvenile dhufish may be detected in the future.

The site surveyed off Castle rock was the shallowest site investigated at only 9 m in depth but had a variety of habitat types and importantly small emergent patches of low profile limestone reef. Again, the higher species diversity was associated with these small patches and species such as breaksea cod and red-lined cardinal fish were recorded on these small patches. The area contained numerous small emergent patches of limestone reef and only a few were surveyed on the dive conducted. It may be possible that a more detailed survey of the area would detect juvenile dhufish associated with these at particular times of the year, as was reported by a reliable recreational diver.

The survey of a large area off Quindalup located some small patches of emergent limestone reef and *Turbinaria* sp. coral habitat types within the seagrass meadows. A high fish species diversity and abundance was associated with these small patches of different habitat within the predominantly seagrass meadows. Such sites may be suitable as recruitment sites for juvenile dhufish but they are small and difficult to locate.

The area south of Rottnest Island was chosen as credible reports of juvenile dhufish occurring in the area were received. Each of the three sites contained a mixture of habitat types particularly the southern site where more sandy habitats were observed, similar to the Perth metropolitan trawl area. Even though no juvenile dhufish were recorded it is likely that juvenile dhufish would occur in this area as the benthic habitat, particularly at the southernmost site, was similar to the Perth metropolitan trawl area where juveniles are known to occur. The current towed video surveys were conducted in poor visibility of only 2 m and the particular site where juveniles were reported is beyond DoF diving limits at 32 m so could not be extensively covered by divers with sDOV. However, it is considered likely that further towed video surveys in this area during better conditions will detect juvenile dhufish.

The survey of the *HMAS Swan* during the current project recorded 27 fish species which is only slightly less than the maximum diversity of 32 species recorded during the initial post-

establishment surveys in December 1999 (Morrison 2007). The initial monitoring of the *HMAS Swan* by Morrison (2003) noted the seasonality of dhufish and also commented on the likely impact of fishing activities in the vicinity of the wreck on the fish community even though there is a 200 m fishing exclusion zone. The ongoing fishing within this exclusion zone was evident during the current survey with a large amount of fishing line observed to be fouled on the rails of the wreck during the dive and a yellow-tail kingfish observed with hooks and a length of leader hanging from its mouth.

The surveys have documented the state of the Quindalup tyre artificial reef in 2012 after more than 20 years since it was deployed. The tyres were detected spread over a large area of 300 x 300 m with some of the pyramid and row structures still intact. However, it was noted that many tyres had broken apart and lay scattered on the seabed. Much of the benthic habitat in the area was bioturbated sand with a few *Posidonia* sp. seagrass beds. It was also noted that there was only a few sessile invertebrates and macroalgae growing on some of the tyres. Despite this a wide diversity of 36 fish species were recorded by all four methods used at the site. The species recorded included the snakeskin wrasse which has been found to occur with juvenile dhufish indicating the site may be suitable for monitoring juvenile dhufish. Of the species few were recreationally targeted fish species with only of a school of silver trevally (*Pseudocaranx dentex*) and a few small samsonfish (*Seriola hippos*). Thus, the suitability of using old tyres as an artificial habitat type for a recreational fishing reef is in question.

4.0 Diet of juvenile dhufish

P. Lewis & J. Dias

4.1 Introduction

WA dhufish are known to undergo a shift in habitat and diet as they reach the size of maturity (Hesp *et al.* 2002, Platell *et al.* 2010). These studies have shown dhufish predominantly consume macro-invertebrates while they are less than 2 years of age and occurring on hard substrate in sandy areas and then shift to a diet of predominantly teleost once the shift to low profile reefs. The analysis of stomach contents can provide evidence of the habitat utilised based on known habitat affinities of consumed prey items. This technique relies on the ability to accurately identify the prey items in the diet and knowledge of the habitat preferences of these preys.

Many diet studies have relied upon traditional gut content analyses. These studies rely on visual examination and hard part analysis of stomach contents to determine the prey species. This technique is restricted in that only recently consumed items can be readily identified and older, more digested items may not be readily identifiable. It is also biased towards crustaceans and fish due to any soft bodied prey items such as worms being quickly broken down and not identifiable. The identification of fish prey relies upon sampling individuals with recently consumed and partially digested prey or the identification from otolith shape. Identification from otolith shape is further restricted in part due to limited otolith catalogues available but also the digestion and erosion of the otoliths with time in the stomach making identification progressively more difficult. The enhancement of traditional visual gut content analyses with complimentary techniques such as genetics are recommended for a full assessment of dietary composition due to high proportions of unidentifiable partially digested contents (Cote *et al.* 2013).

Recent advances in molecular sequencing technology and development of DNA databases have made High Throughput Sequencing (HTS) available for the analysis of complex biological samples such as stomach contents. Molecular techniques are known to be extremely sensitive, being able to amplify very small amounts of DNA and allowing for the detection of an array of prey items which could not possibly be identified by visual or hard part analysis. HTS also has potential to become a cost effective method for the determination of diets due to its high throughput potential. The main restriction with such application of HTS, at present, relates to the still low number of WA's "species barcodes" (diagnostic species-specific DNA fragments) that have been sequenced and made available to the databases. It is therefore likely that many of the DNA barcodes amplified from dhufish diets will not find a match in the database in order to be identified or will not be identified all the way to the species level at this time.

The current study has employed the two techniques of traditional visual examination of stomach contents analysis and HTS in parallel, in an attempt to identify the range of prey items consumed by juvenile dhufish (TL<150 mm). The results, advantages and disadvantages of each of the two techniques are compared and insights provided on the habitat use of juvenile dhufish, based on the habitats used by their prey. The types, variety and quantity of the identified prey along with the habitat associations of these prey consumed will indicate information on the foraging behaviour of juveniles.

4.2 Methods

Juvenile dhufish were sampled at the Augusta main lease site (n=10) and in the trawl ground off the Perth metropolitan area (n=3). The Augusta samples were obtained in February 2012 by gidgee spear while SCUBA diving. The Perth metropolitan samples were caught in March 2012 by fish traps (n=2) and in a small try-net trawl (n=1).

The individuals sampled were frozen immediately and thawed just before processing. Each juvenile was measured (TL, to the nearest mm) and weighed (whole weight g) before the stomach was dissected out with sterile scalpel and tweezers and placed in a new sterile pre-weighed petri dish. The stomachs were cut along the midline and the contents scraped into a petri dish and weighed. Each stomach was given a fullness index from 1 (empty) to 5 (filled to capacity). The contents were irrigated with 0.5-1.0 ml of sterilised de-ionised water to aid in the separation and identification of contents by microscopy.

Stomach contents analysis

The irrigated stomach contents were examined under reflected light using a stereo dissecting microscope (Olympus SMZ745T) fitted with a digital video camera (Jenoptik ProgRes® C7). The contents were gently teased apart using sterile tweezers and dissecting probe with any readily identifiable items, otoliths, invertebrates, etc. and sorted into groups. The items in each group were identified to the lowest taxonomic category possible and counted. Images were captured of all items using image processing software (ProgRes® CapturePro 2.7.6) with a scalebar added to each image for future reference and identification. Once completed, the entire contents of the petri dish was transferred to a sterile vial and frozen at -20°C for molecular analysis.

DNA extraction and sequencing

The stomach contents of six individuals (3 Perth metropolitan and 3 Augusta) were processed using HTS methodologies. Samples were thawed and approximately 1 ml of each stomach content homogenate was transferred to a 2 ml Eppendorf tube, the remaining of all samples being stored at -20°C . Extractions were performed using QIAamp DNA Stool Mini Kit (QIAGEN) as per manufacturer's instructions. DNA was eluted in 50 μL of AE buffer and DNA extracts stored at -20°C until further analysis.

Each extract was screened using real-time PCR and series of primer pairs available at the Murdoch University Ancient DNA research laboratory and from the literature (Table 1). Each extract was amplified at neat, 1:10 and 1:100 dilutions using the ABI Step One Real Time PCR machine. From all the primer pairs tested only the *rbcl*, *trnL*, ZBJ-Art, LCO1490/Uni-MinibarR1 and 16S primer sets generated amplicons. For each sample, the DNA dilution (neat, 1:10 and 1:100) that generated the best compromise of amplicon DNA yield/PCR inhibition was assigned a unique MID-tagged primer set. MID-tagged real-time PCR was carried out and only the MID-tagged primer sets *rbcl*, LCO1490/Uni-MinibarR1 and 16S generated amplicon quantities suitable for HTS.

Table 1. Primer pairs tested for the amplification of marine plant and animal DNA.

Taxonomic Group	Primers		Amplicon size (bp)	Target Region	Target Taxon	Reference
	Forward	Reverse				
Plants	ps bA	trnH	300-450	Plastid tmH-psbA intergenic spacer	seagrass	Budarf <i>et al.</i> 2011
	p23SrV f1	p23SnewR		23s rDNA plastid marker	eukaryotic algae & cyanobacteria	Sherwood & Presting 2007 Clarkston & Saunders 2010
	rbcl fw	rbcl rev	183	chloroplast gene encoding the large subunit of ribulose-biphosphate carboxylase (rbcl)	plants	Poinar <i>et al.</i> 1998
	trnLc	trnLh		trnL (UAA) Group1 intron in chloroplast DNA	plants	Taberlet <i>et al.</i> 2007
	ZBJ-ArtF1c	ZBJ-ArtR2c	100-300	COI barcode	arthropods	Zeale <i>et al.</i> 2010
Animals	LCO1490	HCO2198	710	COI barcode	invertebrates	Folmer <i>et al.</i> 1994
	Uni-MinibarF1	Uni-MinibarR1	120-150	COI barcode	invertebrates	Meusnier <i>et al.</i> 2008
	LCO1490	Uni-MinibarR1	120-150	COI barcode	invertebrates	Folmer <i>et al.</i> 1994 Meusnier <i>et al.</i> 2008
	16S1F degenerate	16S2R degenerate	180-270	mitochondrial 16S rDNA	fish	Deagle <i>et al.</i> 2007

Reactions were conducted in duplicate and amplicons pooled together to minimise the effects of PCR stochasticity. The resultant pooled amplicons were purified using Agencourt AMPure XP PCR Purification Kit (Beckman Coulter Genomics, NSW, Australia), and eluted in 40 µL of buffer. Purified amplicons were electrophoresed on 2% agarose gel and amplicons were pooled in approximately equimolar ratios based on band intensity. All procedures involved in the setup of the sequencing run (emulsion PCR and bead recovery), including the sequencing run itself, were carried out according to the Roche GS FLX Junior (Roche) protocols for amplicon sequencing (<http://www.454.com>).

Sequences were searched using BLASTN against the NCBI GenBank nucleotide database. This was automated in the Internet-based bioinformatics workflow environment, YABI (<https://ccg.murdoch.edu.au/yabi/>). The BLAST results that were obtained using YABI were imported into MEtaGenome Analyzer (MEGAN) where they were taxonomically assigned.

4.3 Results

The 13 stomach content samples used in this study were obtained from small juvenile dhufish of 107-154 mm in TL (Table 4.1), with most (n=9) 130-154 mm in size.

Taxonomic groups

Microscopy

Eight different invertebrates and four fish were identified by visual examination of the juvenile dhufish stomach contents (Table 4.1). Most individuals contained 2-4 different invertebrates and comparison of prey items between the sites indicated that juvenile dhufish sampled in the Perth metropolitan area had a lower variety, with only three types of prey items compared to at least 11 different items identified in the Augusta juveniles (Table 4.1). Only ostracods (*Mydocopa*) and sea lice (*Flabellifera* isopods) were in the stomachs of fish from both Augusta and the Perth metropolitan area. A parasitic fluke worm (*Digenea*) was also found in the stomachs of juveniles from both areas (Figure 4.1).

The two Perth metropolitan juveniles sampled by fish traps had very full stomachs and contained large numbers of sea lice (*Flabellifera*) while these only occurred as single items in three of the Augusta juveniles. The third trawl sampled Perth juvenile only contained ostracods which were also in the other Perth juveniles and four of the Augusta juveniles. None of the Perth juveniles contained fish otoliths while all but one of the Augusta juveniles contained fish otoliths with four pairs of Carangidae sp. otoliths found in one individual. Along with otoliths only one type of shrimp (*Caridae*1) were present in more than half of the samples from Augusta.

The stomachs of four Augusta juveniles contained what appeared to be shell plates and hinged sections of a goose barnacle (Cirripedia: *Lepadomorpha*) (Figure 4.2). These distinctly shaped pieces of shell within a membrane and hinged section between shell plates may be better identified with further investigation.

Table 4.1 Occurrence of prey items identified to Order, Suborder or Genus (Fish) by number in the stomach contents of each individual juvenile dhufish with details of area and size (TL in mm).

	Perth Metropolitan			Augusta									
	Me1	Me2	Me3	Au1	Au2	Au3	Au4	Au5	Au6	Au7	Au8	Au9	Au10
TL (mm)	122	130	132	113	107	143	154	143	147	131	121	142	131
Invertebrates													
Crustacea													
Malacostraca													
Amphipoda													
Gammaridae			6										
Decapoda													
Caridae1				1		1	1			1	1		1
Caridae2													1
Penaeoidea								1					
Isopoda													
Anthuridae					1		1	1					
Flabellifera		35	174			1			1				1
Maxillopoda													
Thoracica													
Lepadomorpha				1	1		1			1			
Ostracoda													
Mydocopa	2	2	2			1		2	1		9		
Trematoda													
Digenea													
Plagiorchiida	2			6	1		1	1		7	2	3	
Total Suborder	2	2	3	3	3	3	4	4	2	3	3	1	3
Vertebrates													
Perciformes													
Silaginidae					2								
Carangidae									2			8	2
Gerreidae						2	3						
Labridae											2		1
Total Genera	0	0	0	0	1	1	1	0	1	0	1	1	2



Figure 4.1 Image of fluke worms (Digenea: Plagiorchiida) found in stomachs of juvenile dhufish sampled from Perth and Augusta regions. Scalebar = 0.5 mm.



Figure 4.2 Image of digested shell plates within membrane and hinge section (to left) possibly from a type of goose barnacle in stomach contents of juvenile dhufish sampled from Augusta. Scalebar 2.0 mm.

Among the fish genera identified from the otoliths included silverbelly (*Parequula melbournensis*) or juvenile dhufish in the stomachs of two of the larger juveniles (TL=143 & 154). The comparison of these otoliths found in the stomachs to those from a larger silverbelly (TL=115 mm) and juvenile dhufish (TL=100 mm) is given in Figure 4.3. The outline is most similar to the silverbelly otoliths although the edges are undulated on those from the stomach. This may be due to uneven erosion of the otoliths in the stomachs or different otolith shape in smaller silverbelly. The sulcal groove in the stomach content otoliths is wider towards the rostrum (to the anterior side of the primordium) than that of the silverbelly and is more similar to dhufish. The posterior section of the sulcal groove in all three types otoliths has a slight bend towards the ventral side although the posterior end of the groove is above the posterior point of the otolith in both the stomach content and silverbelly otoliths. The stomach content otoliths are not an exact match with either and it may be possible that the differences are due to uneven erosion of silverbelly otoliths but they could also be from another species for which the otoliths were not available for comparison. It seems more likely that they are from silverbelly.



Figure 4.3 Images of otoliths from a) the stomach contents of a juvenile dhufish sampled from Augusta, b) silverbelly (TL= 115 mm) and c) juvenile dhufish (TL=100 mm). Scale bars a) = 0.5 mm, b) = 1.0 mm and c) = 1.0 mm.

Molecular analysis

The GS FLX Junior (Roche) sequencing run generated a total of 14520 reads for this study consisting of 7446 reads from the *rbcl* primers, 5961 reads from the 16S primers and 1113 reads from the LCO1490/ Uni-Minibar R1 primers. Sequences were obtained for all dhufish samples. The HTS technique was able to identify four species of fish and six species of invertebrate in the stomach contents of the six individual dhufish analysed (Figure 4.4). The technique was able to identify fish prey in five of the six dhufish samples. A number of reads could not be identified as indicated by the size and colour of the pie charts to the left of the identified reads.

The known distributions of some species identified by HTS to be in the stomach contents of juvenile do not include the Indian Ocean or southern WA (Figure 4.4). It is unlikely that these particular species were in the stomachs but the reads may belong to a similar closely related species that does occur in WA but has not been sequenced. Similarly the unusual detection of other items such as human and morabine grasshopper (*Vandimenella viatica*) DNA in the stomach contents may be due to contamination (samples had been previously handled for hard part analysis) or lack of WA species specific bar codes in the databases.

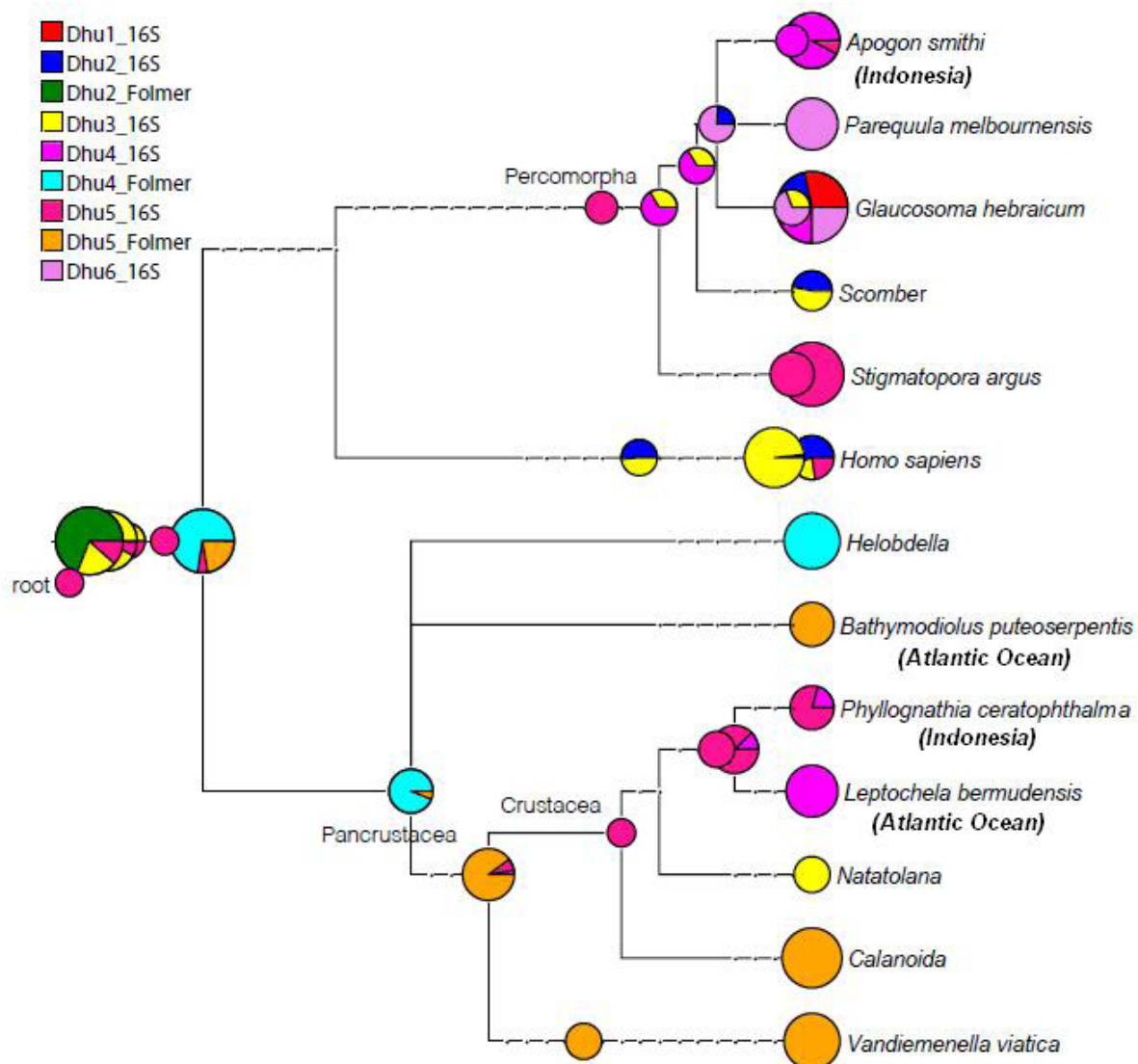


Figure 4.4 Occurrence of prey species in the stomach contents of juvenile dhufish with distribution given below in brackets for those not occurring within southern WA.

Comparison

Overall, the two techniques identified seven different types of fish and 12 different types of invertebrates in the stomach contents of the 13 juvenile dhufish (Tables 4.1 and 4.2).

Comparison of HTS and microscopy results for the six individual samples analysed by both methods indicated only two identifications in common being 1) *Caridae* shrimp in Augusta 1 and 2) *Gerreidae* fish in Augusta 3 (Table 4.2). Both techniques were able to identify a number of prey items not detected by the other method for each individual. The HTS technique could identify items with no remaining visible hard parts in the stomach such as the annelid and scombridae fish sp. The microscopy could identify prey items which were not identified by sequencing such as ostracods and isopods which is likely due to the lack of capability of primers to amplify certain species and/or the absence of species barcodes in the DNA databases, as indicated by the unidentified sequences to the left in Figure 4.4.

Table 4.2. List of prey items to suborder (invertebrates) or family (fish) found in the gut contents of dhufish using visual morphology-based identification and High Throughput Sequencing (HTS) methods.

Sample	TL	Crustacea		Mollusca		Trematoda		Annelida		Perciformes	
		Morph	HTS	Morph	HTS	Morph	HTS	Morph	HTS	Morph	HTS
Perth 1	122	Mydocopa				Plagiorchiida					
Perth 2	130	Mydocopa / Flabellifera	Cymothoida								Scombridae
Perth 3	132	Mydocopa / Flabellifera / Gammaridae									Scombridae
Augusta 1	113	Lepadomorpha/ Caridae	Phyllognathia & Leptochela (Caridae)			Plagiorchiida		Rhynchobdellida			Apogonidae
Augusta 2	107	Lepadomorpha/ Anthuridae	Phyllognathia (Caridae)/ Calanoida			Mytiloida	Plagiorchiida	Sillaginidae		Apogonidae/ Syngnathidae	
Augusta 3	143	Mydocopa / Flabellifera / Caridae						Gerreidae		Gerreidae	

4.4 Discussion

The study has utilised two complimentary techniques to reveal that juvenile dhufish consume a wide variety of prey items. The combined results of the traditional visual identification of gut contents and the analysis of DNA in the gut contents by the HTS techniques identifying 19 different types of prey including seven different fish prey species. The types of prey consumed indicate juvenile dhufish are opportunistic feeders utilising a wide range of available prey including species generally associated with the surrounding sand or seagrass habitats but also some pelagic and sessile benthic invertebrate species. The comparison of diet between juveniles from the Perth metropolitan and Augusta areas indicated a lower diversity in the former and this may reflect the lower habitat diversity in the area and may also be influenced by sampling method. The detection of prey to species level from diets was better accomplished using HTS, although the identifications are not all for species occurring in WA. The two techniques each have their advantages and disadvantages with the HTS technique showing a great potential for use in diet studies once sufficient WA species have been sequenced and entered into available databases and specific primers developed.

The juveniles sampled at the Augusta site had a wide variety of prey in the diet suggesting they are utilising the full range of habitats such as seagrass beds, sand and macroalgal wrack surrounding the Augusta site. The prey items identified were not common to all of the juveniles with most found in less than half of the stomachs indicating the juveniles are not all utilising the same habitats and resource. The fish prey included species such as a banded pipefish (*Stigmatopora argus*) and silverbelly, normally associated with sand and seagrass areas, apogonids which are often associated with low profile reef and scombrids which are generally a more pelagic species. The results also indicated the diet of juvenile dhufish contained a high variety and number of small crustaceans including isopods, amphipods, ostracods and Caridean shrimp which could be associated with the macroalgal wrack. The finding of a type of barnacle in the stomachs of four of the ten juveniles sampled at Augusta was not expected and may possibly be due to it being dislodged by cleaning at the lease and subsequently consumption by juvenile dhufish. Platell *et al.* (2010) also recorded molluscs in the diet of juvenile dhufish, again indicating they are opportunistic feeders.

The juveniles sampled in the Perth metropolitan area (in this study) had a lower variety of prey items in their diet, which were predominantly sand associated crustaceans. The high occurrence of isopods may be due to these juveniles being sampled by overnight baited- traps. The bait in the traps may have attracted the isopods in high numbers. The occurrence of pelagic species such as Scomber sp. of fish and ostracods indicates that juvenile dhufish are feeding in the water column and highly opportunistic feeders.

The stomachs of juvenile dhufish at both sites were found to contain a parasitic flatworm (*Digenea* sp.). More than half, 70%, of the juveniles sampled at the Augusta site were infected by this parasite. Digenean parasites were not reported in a review of dhufish health for aquaculture purposes (Stephens *et al.* 2003) which focussed on gill, liver and external parasites. Digenean parasites have been identified in numerous other marine species such as Sandy Sprat, Samson fish and Tailor in the waters off WA.

The wide variety and types of prey items found in the stomachs of juvenile dhufish in the current study is similar that found in juveniles of less than 150 mm in TL by Platell *et al.* (2010). Both studies found small crustaceans comprised a large component of the diet but there was also evidence for opportunistic feeding on a wide variety of other prey items such as bivalves,

decapods, teleosts and plant material. The current study found a higher occurrence of teleosts in the stomachs of small juvenile dhufish less than 150 mm in TL with eight of the ten Augusta juvenile stomachs containing otoliths compared to less than 20% of similar sized juveniles with teleosts in a previous study (Platell *et al.* 2010).

The comparison of traditional and molecular methods provided somehow similar, but most importantly, complimentary results giving an overall indication of the prey types for juvenile dhufish. Prey items in the same genera were only detected by both methods on only two occasions. The traditional visual identification results are restricted by the degree of digestion of the prey items. None of the fish species could be directly identified by this method due to the degree of digestion. The fish species could only be discerned from the shape of the otoliths, and only one of these to the likely species when not too eroded. Similarly, many of the invertebrates were broken into pieces and not whole making identification difficult. The HTS technique detected evidence of important prey items that indicated habitat use, such as the banded pipefish and Scombrid sp., which were not detected by the visual examination.

The molecular analysis is restricted by the species barcodes in the databases and as a result allocated results to related species which do not occur in WA. Species such as the Indonesian Smith's cardinalfish (*Apogon smithii*) is likely to be identified in the future as one of the locally occurring Apogonidae species such as the redlined cardinalfish (*Apogon victoriae*) which has been observed on the Augusta lease. Other identifications are less obvious but the hydrothermal vent mussel (*Bathymodiolus putoserpentis*) is likely to be the bivalve recorded in the visual analysis and identified as a related locally occurring mussel species in the future. The traditional visual analysis did indicate some species such as the ostracods which were missed in the HTS analysis using current analysis methods and DNA databases.

The HTS results are restricted in their accuracy as many WA species are not sequenced so could not be identified. The results identified the closest related species in the current database. Unfortunately, extensive projects on the barcoding of other groups within the crustacea and molluscs, and that might include the species found in WA constitute a major gap in barcode databases. The unusual detection of land arthropods is most likely an artefact from the lack of WA species specific barcodes in the databases. Similarly the identification four species in the diet of dhufish which are not known to occur in WA, such as the hydrothermal vent mussel, are likely to be due to the lack of WA species in databases and identification attributed to a related species in the database. Barcoding of species endemic to WA are a priority, as they would provide essential background for the successful application of metabarcoding studies using HTS from complex diet samples.

5.0 Recommendations

The extension to the NRM juvenile dhufish has added greatly to our knowledge of the species and given an insight into the potential of using artificial habitats to monitor annual recruitment.

The main recommendations from this extension include;

- The continued collaboration with the lease holder in annually surveying the main abalone aquaculture lease site off Augusta during the November – February period, which will provide valuable information on the annual recruitment strength of juvenile dhufish in this region.
- The ongoing use of stereo video diver survey techniques in annual juvenile dhufish recruitment surveys allowing the analysis of length frequency data for year class modes to be determined.
- The future validation of this annual recruitment index with the age structure data for dhufish catches from the area.
- The development of a project to establish and assess artificial recruitment monitoring sites (ARMS), similar to the “V-pipes” formation at the Augusta lease, at a number of locations along the coast in similar areas of predominantly sandy habitat for not only monitoring annual recruitment but also potentially enhancing localised recruitment.
- The continued assessment of small artificial habitats to monitor dhufish recruitment in the Perth metropolitan area.
- The continued search for natural areas of nursery habitat for juvenile dhufish in future years as the current surveys may be during low recruitment when juveniles are not abundant in the areas surveyed.
- The discontinuation of the large scale trawl, trapping, and BRUV surveys as an index of dhufish recruitment in the Perth metropolitan area.
- The advancement of the genetic HTS technique by populating databases with barcodes of WA species and development of specific primers for marine plants and relevant animal groups for future studies of species endemic to WA.

6.0 Acknowledgements

The authors would like to acknowledge the funding provided for this project by the Natural Resource Management group. The project has relied upon assistance with fieldwork from the Finfish, Mollusc and Biodiversity research groups at the Department of Fisheries and their help is greatly appreciated. The project would not have been a success without the assistance and contributions of Brad Adams at Ocean Grown Abalone in Augusta. Next generation analyses of stomach content samples were performed by Joana Dias (DoF) and researchers (Nicole White, James Haile and Mike Bunce) at Murdoch University. Finally the author wishes to thank the reviewers of this report who provided invaluable feedback.

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Appendix 1 Summary of species recorded in Perth metropolitan trawl area during 2012 by method.

Family	Genus	Species	BRUV	Fish traps	Trawl
Callionymidae	<i>Callionymus</i>	<i>goodladi</i>			10
Carangidae	<i>Carangidae</i>	sp.			135
	<i>Pseudocaranx</i>	<i>dentex</i>		68	2
		sp	26		
		<i>wrightii</i>			525
	<i>Seriola</i>	<i>hippos</i>	2		1
	<i>Trachurus</i>	<i>novaezelandiae</i>	93	1	31
Chaetodontidae	<i>Chaetodon</i>	<i>assarius</i>	1		
Clupidae	<i>Sardinella</i>	<i>lemuru</i>			1
Cynoglossidae	<i>Cynoglossus</i>	<i>broadhursti</i>			1
	<i>Paraplagusia</i>	<i>bilineata</i>			2
Dasyatidae	<i>Dasyatis</i>	<i>brevicaudata</i>	1		
Enoplosidae	<i>Enoplosus</i>	<i>armatus</i>		2	
Gerridae	<i>Parequula</i>	<i>melbournensis</i>	14	3	80
Glaucosomatidae	<i>Glaucosoma</i>	<i>hebraicum</i>	1	3	1
Gonorynchidae	<i>Gonorynchus</i>	<i>greyi</i>			2
Haemulidae	<i>Plectorhinchus</i>	<i>flavomaculatus</i>		1	
Harpadontidae	<i>Saurida</i>	<i>undosquamis</i>			39
Heterodontidae	<i>Heterodontus</i>	<i>portusjacksoni</i>	1		2
Kyphosidae	<i>Microcanthus</i>	<i>strigatus</i>		3	1
Labridae	<i>Coris</i>	<i>auricularis</i>	26	48	7
	<i>Ophthalmolepis</i>	<i>lineolatus</i>	1		
	<i>Scobinichthys</i>	<i>granulatus</i>		2	3
Monacanthidae	<i>Acanthaluteres</i>	<i>spilomelanurus</i>			7
	<i>Brachaluteres</i>	<i>jacksonianus</i>			3
	<i>Chaetodermis</i>	<i>pencilligera</i>			10
	<i>Meuschenia</i>	<i>hippocrepis</i>		1	
	<i>Monacanthus</i>	<i>chinensis</i>			6
	<i>Nelusetta</i>	<i>ayraudi</i>			2
Mullidae	<i>Parupeneus</i>	<i>chrysopleuron</i>	4		39
	<i>Upeneichthys</i>	<i>vlamingii</i>		5	1
		<i>lineatus</i>			7
	<i>Upeneus</i>	<i>asymmetricus</i>	1		396
Myliobatidae	<i>Myliobatis</i>	<i>australis</i>	2		1
Nemipteridae	<i>Pentapodus</i>	<i>vitta</i>	2	2	2
Ostraciidae	<i>Anoplocapros</i>	<i>robustus</i>			8
	<i>Aracana</i>	<i>aurita</i>			1

Family	Genus	Species	BRUV	Fish traps	Trawl
	<i>Lactoria</i>	<i>concatenatus</i>			45
Pempheridae	<i>Pempheris</i>	<i>klunzingeri</i>		10	11
Platycephalidae	<i>Platycephalus</i>	<i>bassenis</i>			1
		<i>longispinis</i>	10	8	131
Pleuronectidae	<i>Ammotretis</i>	<i>elongatus</i>			10
Rhinobatidae	<i>Aptychotrema</i>	<i>vincentiana</i>	1		5
	<i>Trygonorhina</i>	<i>fasciata</i>	2	2	3
Scorpaenidae	<i>Maxillicosta</i>	<i>scabriceps</i>			8
	<i>Neosebastes</i>	<i>pandus</i>			2
	<i>Pterois</i>	<i>volitans</i>			1
	<i>Scorpaena</i>	<i>sumptuosa</i>			2
Scorpididae	<i>Neatypus</i>	<i>obliquus</i>	3	8	2
Serranidae	<i>Caesioscorpis</i>	sp.		1	2
Sillaginidae	<i>Sillago</i>	sp.	10		
	<i>Sillago</i>	<i>bassensis</i>		3	27
		<i>robusta</i>			508
Soleidae	<i>Phyllichthys</i>	<i>punctatus</i>			1
	<i>Strabozebrias</i>	<i>cancellatus</i>			2
Sparidae	<i>Chrysophrys</i>	<i>auratus</i>	1	83	
	<i>Rhabdosargus</i>	<i>sarba</i>	2	2	
Sphyracidae	<i>Sphyracna</i>	<i>novaehollandiae</i>			3
Squatinae	<i>Squatina</i>	<i>australis</i>			3
Synodontidae	<i>Trachinocephalus</i>	<i>myops</i>			4
Terapontidae	<i>Pelsartia</i>	<i>humeralis</i>		12	
Tetradontidae	<i>Lagocephalus</i>	<i>sceleratus</i>	3	1	
Tetraodontidae	<i>Polyspina</i>	<i>piosae</i>		1	6
	<i>Torquigener</i>	<i>vicinus</i>	3		129
Triakidae	<i>Mustelus</i>	<i>antarcticus</i>		1	
Triglidae	<i>Chelidonichthys</i>	<i>kumu</i>			25
Uranoscopidae	<i>Kathetosoma</i>	<i>laeve</i>			1
Urolophidae	<i>Urolophus</i>	<i>mucosus</i>			293
Veliferidae	<i>Velifer</i>	<i>multiradiatus</i>			86
		Count	24	26	57

Appendix 2 Summary of fish species recorded at additional sites.

Family	Genus	Species	Bunbury	Castle Rock	Dunsborough	HMAS Swan	Quindalup	South Rottne	Tyre reef
Apogonidae	<i>Apogon</i>	<i>victoriae</i>	1	3					
Carangidae	<i>Pseudocaranx</i>	<i>dentex</i>						3	42
		sp.	1			9	2	1	
	<i>Seriola</i>	<i>hippos</i>				9	6		5
		<i>lalandi</i>				8			
Chaetodontidae	<i>Chaetodermis</i>	<i>penicilligera</i>							2
	<i>Chaetodon</i>	<i>assarius</i>						1	1
	<i>Chelmonops</i>	<i>curiosus</i>			1	8	1		2
Cheilodactylidae	<i>Cheilodactylus</i>	<i>gibbosus</i>				2	1		
	<i>Dactylophora</i>	<i>nigricans</i>					1		
Dasyatidae	<i>Dasyatis</i>	<i>brevicaudata</i>					3	1	4
Diodontidae	<i>Diodon</i>	<i>nicthemerus</i>	1			101			1
Enoplosidae	<i>Enoplosus</i>	<i>armatus</i>					2	2	2
Gerridae	<i>Parequula</i>	<i>melbournensis</i>		7			127	2	4
Girellidae	<i>Girella</i>	<i>zebra</i>		1					
Glaucosomatidae	<i>Glaucosoma</i>	<i>hebraicum</i>				1	1		
Heterodontidae	<i>Heterodontus</i>	<i>portusjacksoni</i>					1	3	1
Labridae	<i>Achoerodus</i>	<i>gouldii</i>				1			
	<i>Anampses</i>	<i>geographicus</i>				1			
	<i>Austrolabrus</i>	<i>maculatus</i>	1	1	1	3	3	2	7
	<i>Bodianus</i>	<i>frenchii</i>					1	1	
	<i>Choerodon</i>	<i>rubescens</i>	1				1		
	<i>Coris</i>	<i>auricularis</i>		7	1	10	61	58	100
	<i>Eupetrichthys</i>	<i>angustipes</i>				1	1		1
	<i>Labroides</i>	<i>dimidiatus</i>					1		
	<i>Notolabrus</i>	<i>parilus</i>	1	3		7	9	1	4
	<i>Ophthalmolepis</i>	<i>lineolatus</i>	1	6			6	4	3
	<i>Pictilabrus</i>	<i>laticlavus</i>		1			1		
	<i>Pseudolabrus</i>	<i>biserialis</i>	1			3	2	3	
	<i>Scobinichthys</i>	<i>granulatus</i>					8		1
Monacanthidae	<i>Acanthaluteres</i>	<i>spilomelanurus</i>	1	1	1		196		
	<i>Eubalichthys</i>	<i>mosaicus</i>				1			
	<i>Meuschenia</i>	<i>flavolineata</i>				5	3		2
		<i>galii</i>							1
		<i>hippocrepis</i>							1
		<i>venusta</i>						1	
	<i>Monacanthidae</i>	sp.	1						
Mullidae	<i>Parupeneus</i>	sp.						1	
	<i>Upeneichthys</i>	sp.	1						
		<i>vlamingii</i>	1		1		18		6
Muraenidae	<i>Gymnothorax</i>	<i>woodwardi</i>						1	
Myliobatidae	<i>Myliobatis</i>	<i>australis</i>	1		1		3		1

Family	Genus	Species	Bunbury	Castle Rock	Dunsborough	HMAS Swan	Quindalup	South Rottne	Tyre reef
Odacidae	<i>Neoodax</i>	<i>balteatus</i>		1			1		1
Orectolobidae	<i>Orectolobus</i>	sp.						1	
Ostraciidae	<i>Anoplocapros</i>	<i>amygdaloides</i>			1				
		<i>lenticularis</i>					1		1
		sp.							5
	<i>Aracana</i>	<i>aurita</i>				2	1		
Pempheridae	<i>Pempheris</i>	<i>klunzingeri</i>	1			1	100	2	16
Pentacerotidae	<i>Paristiopterus</i>	<i>gallipavo</i>					2		1
Plesiopidae	<i>Paraplesiops</i>	<i>meleagris</i>					1		1
	<i>Trachinops</i>	<i>noarlungae</i>			1		120		60
Plotosidae	<i>Paraplotosus</i>	<i>albilabris</i>					2		
Pomacentridae	<i>Chromis</i>	<i>klunzingeri</i>		12		4	66	6	59
		sp.							15
		<i>westaustralis</i>						23	
	<i>Parma</i>	<i>mccullochi</i>				10			
Rhinobatidae	<i>Trygonorrhina</i>	<i>fasciata</i>			1		8	3	
Rhynchobatidae	<i>Rhynchobatus</i>	sp.					1		
Scorpaenidae	<i>Neosebastes</i>	<i>pandus</i>					2		
	<i>Scorpaena</i>	<i>sumptuosa</i>					1		1
Scorpididae	<i>Neatypus</i>	<i>obliquus</i>				5		121	3
	<i>Scorpi</i>	<i>aequipinnis</i>				1			
	<i>Tilodon</i>	<i>sexfasciatus</i>				7			1
Serranidae	<i>Caesioperca</i>	<i>rasor</i>						1	
		sp.						4	
	<i>Caesiocorpi</i>	sp.						20	
	<i>Epinephelides</i>	<i>armatus</i>		1			3		1
	<i>Hypoplectrodes</i>	<i>nigroruber</i>	1			1	1		1
Sparidae	<i>Chyrosophrys</i>	<i>auratus</i>				7	2	1	
	<i>Rhabdosargus</i>	<i>sarba</i>				109			
Sphyraenidae	<i>Sphyraena</i>	sp.	1						
Terapontidae	<i>Pelsartia</i>	<i>humeralis</i>					1		
Tetradontidae	<i>Lagocephalus</i>	<i>sceleratus</i>					1		
	<i>Omegophora</i>	<i>armilla</i>							1
		<i>cyanopunctata</i>				1			
Triakidae	<i>Mustelus</i>	<i>antarcticus</i>					1		
Urolophidae	<i>Urolophus</i>	sp.						1	
	<i>Urolophus</i>	<i>circularis</i>					1		
		sp.							2
	<i>Urolophus</i>	<i>expansus</i>			1				
		<i>westraliensis</i>			1				
Invertebrates	<i>Octopus</i>	<i>tetricus</i>					2	1	
	<i>Squid</i>				1				
Total number of species			16	12	12	27	46	28	36