

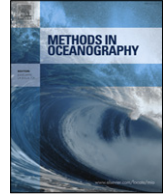


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Assessing pelagic fish populations: The application of demersal video techniques to the mid-water environment



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ABSTRACT

In the open ocean, the movements and habitat use of large mobile predators are driven by dynamic interactions between biological and physical variables and complex predator–prey relationships. Understanding the spatial and temporal distributions of pelagic fishes and sharks is a critical component of conservation and fisheries management. Here, we report on a novel non-extractive method for the study of pelagic wildlife, based on baited stereo-camera rigs. The mid-water rigs were derived from existing methodology commonly used in demersal fish surveys. We present new data from 66 moored deployments in Shark Bay, Western Australia (26°10'S, 113°06'E) in seabed depths of up to 60 m as a demonstration of the rigs' ability to resolve spatial variability in pelagic fish and shark assemblages, and to make accurate stereo-measurements of animal lengths. We observed 248 pelagic

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fishes and sharks from 27 species and 10 families and were able to distinguish between assemblages based nominally on location. We make some general recommendations on optimal deployment protocols and sampling effort regimes, based upon species accumulation rates and times of Max N (maximum number of individuals of a given species in a single video frame). Regression analyses between high quality and low quality stereo-measurements of fish fork-lengths and range were highly significant, indicating that body lengths and distance estimates were consistent even when stereo-measurements were deemed of low quality. Mid-water stereo-video camera rigs represent an efficient tool for the rapid and non-extractive monitoring of pelagic fish and shark populations, with particular relevance for application in no-take marine protected areas.

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1. Introduction

Following the overexploitation of a number of coastal fish species in the 20th century (Jackson et al., 2001), many populations of now threatened pelagic fish and sharks have collapsed (Dulvy et al., 2008; Myers and Worm, 2003; Clarke et al., 2006; Collette et al., 2011). Declines in pelagic fish and shark numbers (henceforth referred to as ‘pelagics’) have been a source of debate, in part because of difficulties in managing high-seas fisheries (Sumaila et al., 2007; Cullis-Suzuki and Pauly, 2010), but also because the lack of fishery independent time series data impairs the assessment and quantification of long-term trends in abundance (Myers and Worm, 2003; MacKenzie et al., 2009; Polacheck, 2006; Sibert et al., 2006; Hampton et al., 2005). Commercial catch statistics have several limitations (Schnute and Richards, 2001), such as temporally and spatially uneven sampling effort, discrepancies in gear efficiency, and imprecise reporting. Further challenges associated with the robust sampling of pelagics relate to the animals’ low overall densities (Ramirez-Llodra et al., 2010), their temporally and spatially heterogeneous distributions (Graves, 1996), and complex associations with seabed topography (Worm et al., 2003; Morato et al., 2010).

Scientific tagging efforts have provided a wealth of knowledge on the movements of large marine predators, allowing the investigation of habitat use by a variety of species over both meso- (Brill et al., 1999) and ocean-wide scales (Block et al., 2011, 2005). However, such studies are invasive, and can be expensive (e.g. satellite tags may cost up to US\$5000 each), and logistically challenging, particularly with regards to animal handling. Active acoustic biomass assessments are becoming widespread in fishery-related surveys (MacLennan, 1992) as they can provide information on the abundance and biomass of pelagic assemblages. These techniques have particular vessel requirements such as low noise outputs (Fernandes et al., 2000), and they do not typically provide accurate information on species composition, particularly when diversity is high. Moreover, the low densities of popular commercial species typically encountered in open waters (for example 1.3 yellowfin tuna per km², Bertrand and Josse, 2000) means that acoustic surveys of pelagics are often conducted around fish aggregation devices (FADs, Doray et al., 2009; Josse et al., 1999; Moreno et al., 2007), which are themselves subject to high temporal variability (Doray et al., 2009). As FADs act as ‘ecological traps’ by aggregating pelagics (Girard et al., 2004), populations may appear stable due to continuous replenishment from neighbouring waters (‘hyperstability’) (Hilborn and Walters, 1992) and the large catchment area (10 km diameter, see Dagorn et al. (2010)) inherent to FADs may be inappropriate for detecting local trends in animal abundance.

Large marine protected areas (>10,000 km; MPAs), where fishing activities are restricted to varying degrees, offer broad protection for marine biodiversity and are increasingly being advocated as refuges for pelagics (Sumaila et al., 2007; Koldewey et al., 2010; Game et al., 2009). Consequently, there is a need for effective and non-invasive monitoring of pelagics such that the benefits of MPAs with respect to population recovery or maintenance (in the case of healthy populations) can be

evaluated (Kaplan et al., 2010). Baited camera surveys, initially developed for use in the deep sea (Isaac and Schwarzlose, 1975), are becoming increasingly popular as a non-invasive method for sampling marine systems, and have been successfully applied in fragile habitats and marine sanctuaries at a range of depths (Watson et al., 2007; Heagney et al., 2007; McLean et al., 2010).

Baited camera surveys have a number of attributes that render them promising in the context of pelagic monitoring. Firstly, they are more effective at sampling predators than underwater visual surveys (using SCUBA divers, see Langlois et al. (2010)). Baited camera surveys may thus be of particular use in pelagic systems, where most fish are shy secondary or higher-level consumers, and actively avoid divers, making underwater visual surveys impractical. Secondly, they are non-extractive and can be validated against extractive methods such as long-line surveys, trawls and trap fisheries (Brooks et al., 2011; Cappelletti et al., 2004; Langlois et al., 2012; Newman et al., 2011). The relatively quick deployment time means that it is possible to generate several samples with multiple baited camera rigs. This latter attribute is particularly conducive to sampling pelagic species exhibiting low population densities and heterogeneous distributions where sampling effort needs to be maximised. Thirdly, relative abundance is assessed using Max N and offers a conservative estimate of abundance. Max N is calculated as the maximum number of animals of a given species observed in a given frame of video (Bailey et al., 2007) (effectively avoiding double counts) and has been shown to be robust through time (McLean et al., 2011) and space (Langlois et al., 2012). Fourthly, other important metrics can be derived from baited camera surveys, such as the time of first arrival TFA (Priode and Merrett, 1996), a highly sensitive abundance proxy when monitoring low-abundance species that has been correlated with catch per unit effort (CPUE)-derived abundance (Priode and Merrett, 1996). Finally, the stereo camera configuration allows individual body length estimates to be produced for many of the observed animals (Harvey and Shortis, 1996), which can be used to determine length-derived demographic metrics such as age structure.

Here we present a new stereo mid-water video camera system, which relies on inexpensive, off-the-shelf, GoPro cameras. The study was motivated by the need to develop a non-extractive method for monitoring the abundance of pelagic species and generating accurate lengths measurements, both of which are key attributes of population and assemblage structure. The overall aim was to develop a technique that would produce data directly relevant to the implementation and monitoring of pelagics in large MPAs.

In our field trials in Shark Bay, Western Australia, we aimed to demonstrate the utility of the method by:

- (1) detecting differences in pelagic fish assemblages across locations;
- (2) assessing the performance of GoPro cameras with respect to measurements length and fish range;
- (3) presenting generic recommendations on deployment duration and sampling effort based upon species richness and Max N accumulation curves for future mid-water camera deployments.

2. Material and methods

2.1. Study site

Shark Bay, Western Australia, (25°30'S 113°30'E) is a large embayment nestled between the Australian mainland and Dirk Hartog Island, approximately 800 km north of Perth (Fig. 1). The bay provides sheltered habitats in an otherwise dynamic and exposed coastline, and has the largest known area of seagrass beds/meadows in the world (Wirsing et al., 2007). The embayment is shallow (average depth ~10 m) and constitutes a hotspot for charismatic wildlife, including birds, dugongs, turtles, migrating humpback whales, bottlenose dolphins and numerous shark species. Strong currents and steep bathymetry gradients render the bay's southern passage and the islands' western slope suitable habitats for large pelagics, such as sharks, tunas, and mackerels.

2.2. Rig design and deployment

Each mid-water camera rig consists of a stainless steel frame (height 1400 mm, base bar 960 mm, Fig. 2), and a pair of GoPro HDHero2 cameras in stereo configuration and encased in Backscatter flat

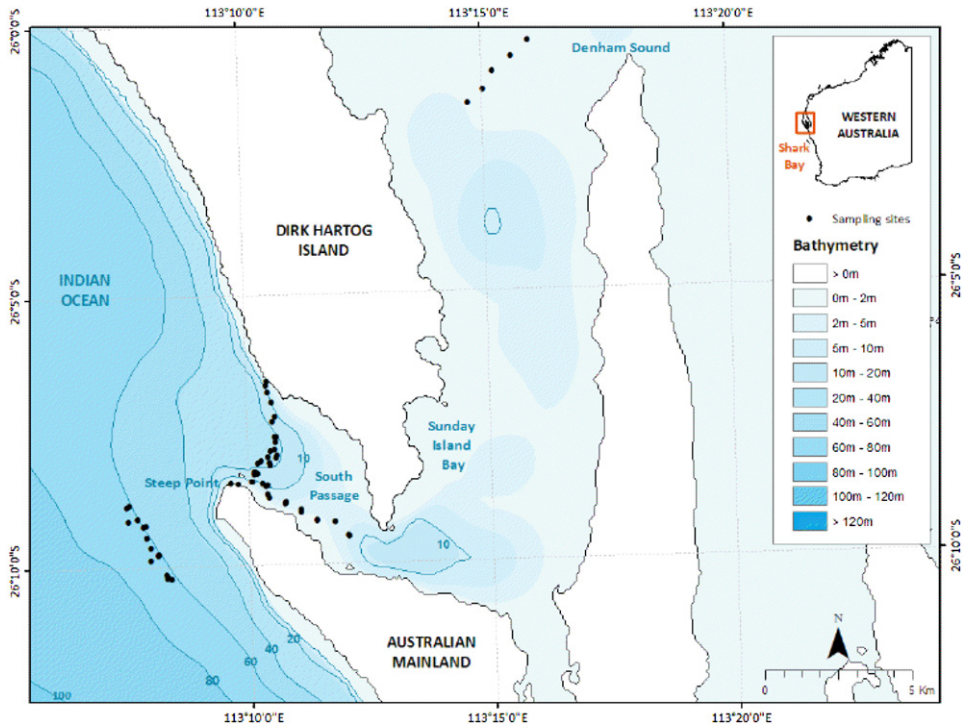


Fig. 1. Sampling stations in Shark Bay, Western Australia. Sampling was conducted along three transects following the westward 60-m isobath, in a north to south direction (Ocean location), along the 10-m and 20-m isobaths at the entrance of South Passage, in a north to south direction (South Passage location), and inside South Passage, in a north-west to south-east direction (Sandy Bay location).

port underwater housings. A complete rig weighs approximately 12 kg. Housings are bolted to the base bar 800 mm apart, with each camera having an inward convergence angle of 4° to allow for an optimised field of view of up to 10 m (Priiede and Merrett, 1996). A bait arm (1.2 m) sits horizontally through the centre of the frame, and serves to hold a bait chamber at a designated and adjustable distance from the cameras. The arm also functions as a sea-anchor and stabilises the rig when moored in moving water, aligning it in the current and limiting the rotation of the rig such that the cameras point downstream.

In this study, each mid-water camera rig was moored with an anchor, an anchor line, surface floats, and an individual tether line to the rig (Fig. 2). A number of different mooring configurations were trialled where variations on the rig suspension and weight systems were tested to reduce camera shake/bounce and maximise footage quality (Supplementary material A can be found online at <http://dx.doi.org/10.1016/j.mio.2013.11.003>). With current camera housing pressure ratings, deployment depth is limited to 55 m but sampling can occur at any depth within this range by adjusting the length of the tether line. Typical deployment time per mooring was 5 min.

2.2.1. Experimental design

A total of 66 mid-water camera deployments were made between the 18th and 23rd of April 2012, between 9 am and 2 pm. Deployment duration ranged between 120 and 135 min. Most deployments occurred along the 20-m isobaths just outside South Passage ($n = 29$), followed by deployments at the Ocean location ($n = 18$) and Sandy Bay ($n = 14$) (Fig. 1). A few experimental deployments were conducted within the Gulf itself ($n = 5$). Several bait types were also tested as attractants: 39 deployments used pilchards (*Sardinops agax*) and squid (hereafter PS) in a suspended bait bag, and 27 deployments were made with a combination of pilchards, squid and a “slurry” (PSS) in a perforated

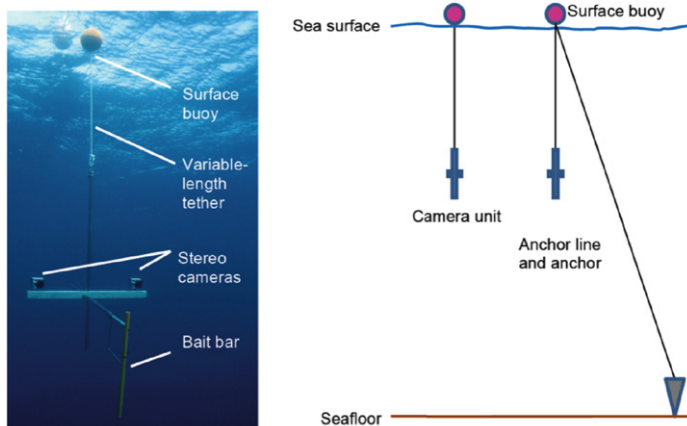


Fig. 2. An individual mid-water camera (left panel, bait is absent in this image), and a schematic representation of free-floating and moored deployment configurations (right panel). See Supplementary material A for overview of various configurations.

container suspended from the bait arm. The slurry consisted of a highly liquidised bait mixture of pilchards and squid. The pilchard to squid ratio was approximately 1:1 in both PS and PSS attractants. The cameras were suspended at 5 m below the sea surface with the exception of the deployments at the Ocean site ($n = 8$ at 10 m, $n = 5$ at 20 m, and $n = 5$ at 40 m).

2.2.2. Video analysis, metrics, and stereo-measurements

All measurements were completed using the EventMeasure software package (www.seagis.com.au). Video analysis commenced at the point when the rig stabilised at its sampling depth and continued for 120 min. Individual animals captured on camera were identified to the lowest taxonomic level possible. The time of first arrive (TFA), maximum individual in a frame of a given species (Max N), and time of Max N were recorded. Camera calibrations were performed independently in an enclosed pool environment for each mid-water rig, enabling stereo photogrammetry of fork length and distance-from-camera (range) to be undertaken on the resulting footage (Harvey and Shortis, 1996). Synchronisation of the two videos was achieved using a clapper-board within the field of view of both cameras prior to deployment. We used synthetic targets of known lengths (473.20 and 764.00 mm, the lengths of the calibration bar) to test the accuracy of the stereo camera systems following calibration.

2.2.3. Statistical analysis

We analysed the effects of location (South Passage (SP), Ocean (O), and Sandy Bay (SB)) on both univariate (species richness and Max N and multivariate (assemblage composition) metrics. The effect of different attractant type (PS, PSS) was considered as a separate factor. For the univariate case, Euclidean distance matrices were calculated and permutational ANOVA used to test for differences among locations and between attractants. As the data were not orthogonal (i.e. only PS was used as an attractant in the Ocean (O) location), the effects of location and attractants were tested separately. Attractants was tested within locations where both attractants were present (SP and SB). Permutational ANOVA was applied also on the multivariate species assemblage data, as a function of location and attractant. The data were counts and included joint zeros, so the Bray–Curtis resemblance matrix was calculated. A canonical analysis of principal coordinates (CAP) Anderson and Willis (2003) was conducted to constrain and visualise the ordination of the species compositional data in multidimensional space. All of these analyses, as well as the calculation of the species accumulation curves (Ugland et al., 2003), were carried out in the software PRIMER v6.0 (Clarke and Gorley, 2006).

Measurement accuracy, based on calibrations prior to sampling, is estimated by root mean square (RMS, in mm) values which are computed for each end point of the length measurements (fork length,

the head and tail position of the target fish) (www.seagis.com.au). RMS error takes the difference between the observed values and estimated values (as predicted for the stereo video) for each end point of the object to be measured (x - y - z coordinates, as defined by the users in Eventmeasure), squares it, determines the mean square, and then finds the root of the resulting value (Hyndman and Koehler, 2006). The relationship between the observed value ($\hat{\theta}$) and estimated value (θ) can be expressed by Eq. (1).

$$RMS(\hat{\theta}) = \sqrt{E((\hat{\theta} - \theta)^2)}. \quad (1)$$

Conventionally, measurements that generate RMS values less than 20 are considered to be accurate (www.seagis.com.au). Length measurements at distances greater than 8 m may also lead to deterioration in RMS values and are typically excluded. The individual lengths and range measurements were classed into two groups: $RMS \leq 20$ (RMS_L) and $RMS > 20$ (RMS_H) where the subscripts L and H refer to “low” and “high” respectively. For each species for which length and range estimates were available for both groups (RMS_L and RMS_H), we calculated the mean length and mean range for each group. Mean values were log 10-transformed to stabilise variance (Zar, 1975) and values of log 10 (RMS_H) for length and range were regressed on the corresponding values for log 10 (RMS_L), reflecting our assumption that the RMS_L values are a more accurate representation of lengths and ranges. Our hypothesis was that the slopes and intercepts of these relationships were equal to 1 and 0 respectively, indicating no difference between the mean estimates of length and range as a function of RMS values. This regression assumes the same length in the fish at RMS_L and RMS_H . We made also multiple measurements of individuals representing eight species across the observed length range (approx. 50 mm to greater than 1.5 m) and calculated the coefficient of variation to assess variability in repeated measures. Finally, to assess whether RMS varied systematically as a function of camera position (individual rig being used), a Chi-Square goodness of fit test determined whether the number of RMS_H measurements were evenly distributed across the five rigs.

3. Results

A total of 761 fishes and sharks were observed on the 66 deployments. Of these, 248 (33%) individuals were classified as pelagic species and 513 (67%) were classified as demersal, based on descriptions from the online FishBase repository (Froese and Pauly, 2011). This sample represented ten families and 27 species of pelagic fishes and sharks and 19 families and 31 species of demersal fishes (supplementary material B1 can be found online at <http://dx.doi.org/10.1016/j.mio.2013.11.003>). Of the 761 fishes, 34 individuals (4.6%) were only identified to family level with almost all of these (94%) being pelagics (supplementary material B2 can be found online at <http://dx.doi.org/10.1016/j.mio.2013.11.003>). Lengths were measured for 107 individuals (43% of observed pelagics), representing ten families and 22 species. Species where individuals maintained too great a distance to the camera to allow for any length measurements including the sharks *Carcharhinus cautus*, *Carcharhinus limbatus*, *Galeocerdo cuvier*, and *Rhizoprionodon acutus*, and the golden trevally *Gnathanodon speciosus*.

3.1. Presence/absence of pelagic species by location and attractant

Pelagic species were observed on 70% ($n = 46$) of deployments. In terms of presence/absence, pelagic species occurred in a similar proportion of deployments at the Ocean (72.2%), South Passage (71.4%), and Sandy Bay (69.0%) locations, but were marginally less common in the exploratory sites in the Gulf (60%). In terms of attractant type, 71% of deployments with PS and 67% of the deployments with PSS attracted pelagic species, consistent with the overall average.

3.2. Species richness, abundance and time of arrival

The number of pelagic species per deployment varied between zero and six, with an average of 1.7 (SD = 1.63) species. The total abundance of pelagic fishes and sharks varied between zero and 26 with a mean value of 3.03 (SD = 4.22, Fig. 4(B)) individuals per deployment. Considering only

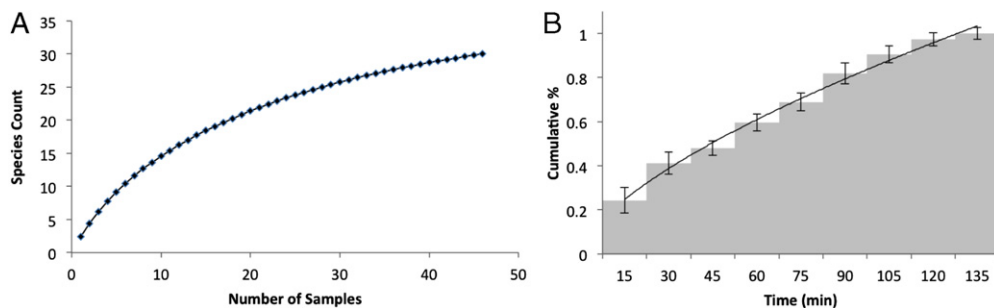


Fig. 3. (A) Species accumulation curve for all samples, (B) and cumulative proportion \pm SD of species per 15 min of video footage, across all samples, with fitted smooth ($y = 0.24x^{0.65}$, $R^2 = 0.99$).

those deployments where pelagic species were present ($n = 46$), the mean number of species per deployment was 2.4 (SD = 1.4) and the mean total abundance per deployment was 4.3 (SD = 4.5). Permutational ANOVA indicated that neither species richness nor abundance varied significantly with location (O, SP and SB; $p = 0.43$ and $p = 0.88$ respectively) or attractant (PS and PSS; $p = 0.53$ and $p = 0.31$ respectively).

All samples were used for the species accumulation curves and time analyses, given only a very small proportion of samples (<15%) had truncated video lengths due to problems with memory cards (<120 min), and because their inclusion did not influence the overall patterns observed. Similarly, we used time of Max N rather than TFA as these two times were coincident for 93% of observations. Species accumulation curves suggested that the number of new species was still increasing with approximately 50 samples (Fig. 3(A)). An analysis of the percentage of species per deployment as a function of time of Max N suggested that approximately 80% of the species observed on a given deployment arrived within the first 90 min (Fig. 3(B)). Time of Max N varied across species with 20% of them arriving within the first hour of deployment, 33% between 30 and 60 min, 37% between 60 and 90 min and only 7% in the final 30 min of footage (Fig. 4(A)). There were no apparent patterns between time of Max N and Max N . The blacktip shark (*Carcharhinus limbatus*; $n = 2$) and sandbar shark (*Carcharhinus plumbeus*; $n = 2$) were among the fastest arrivals with Max N recorded within 5 min of camera deployment while the slender longtom (*Strongylura leiura*; $n = 2$) and the shark mackerel (*Grammatorcynus bicarinatus*; $n = 2$) were the latest to be recorded (arriving at 105 and 120 min, Fig. 4(B)).

3.3. Pelagic species composition

The composition of the pelagic assemblage differed by location (PERMANOVA: $p = 0.002$). Specifically, the Ocean location differed from South Passage ($p = 0.002$) and Sandy Bay ($p = 0.001$) with no significant differences detected between the latter two locations ($p = 0.12$, Fig. 5). The Ocean location was characterised by relatively abundant longfin trevally (*Carangoides armatus*, Fig. 6), distinguishing it from both South Passage and Sandy Bay. South Passage had relatively high abundances of silver toadfish (*Lagocephalus sceleratus*) while Sandy Bay was characterised by relatively high numbers of narrow-banded Spanish mackerel (*Scomberomorus commerson*, Fig. 6). There was no effect of attractant within the SP and SB sites ($p = 0.73$ and $p = 0.69$ respectively).

3.4. Length and range measurements

The comparison of estimated lengths derived from the GoPro stereo rigs and known synthetic lengths (calibrated prior to the survey) indicated a mean length measurement difference of 4.81 mm (min = 0.33, max = 10.2 mm) and 9.4 mm (min = 1.03, max = 22.2 mm) for the 473.2 mm and the 764 mm calibrations respectively. Lengths and range measurements were generated for 105 individual animals, or 42% of the 248 identified and enumerated pelagics. These individuals

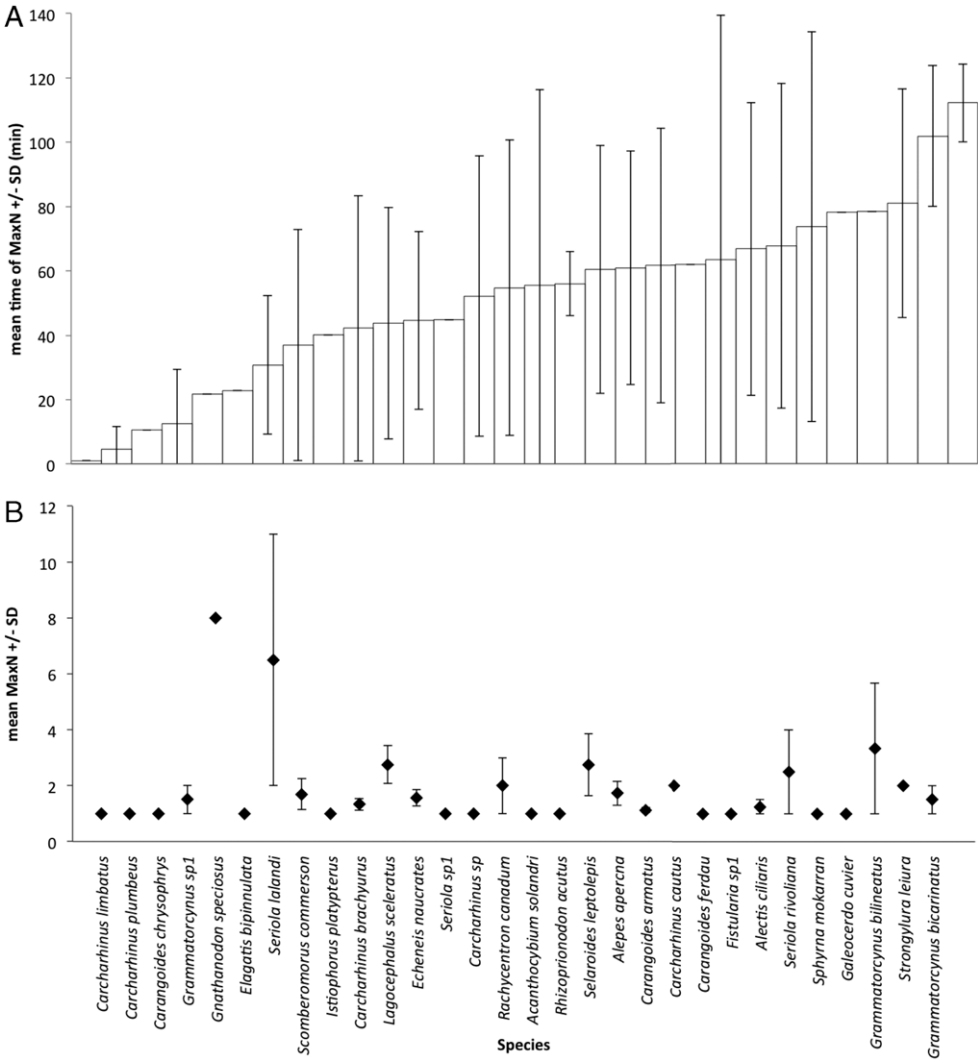


Fig. 4. Mean time of Max N (\pm SD) (A), and mean Max N \pm SD (B) by species.

represented 78% of the 27 species for which Max N estimates were recorded. Length varied from a 38 mm juvenile longfin trevally (*Carangoides armatus*) to a 1.55 m Indo-Pacific sailfish (*Istiophorus platypterus*). RMS values ranged from 0.5 to 97.9, with a median value of 29.9. Of these measurements, 66% exceeded the conventional RMS cutoff of 20 and were coded as RMS_H, with the remaining 34% of measurements coded as RMS_L. There were eight species for which length and range estimates existed for both the RMS_H and RMS_L groups. The regressions between the mean values for the RMS_H and RMS_L groups for both length and range were highly significant ($p = 2.44 \times 10^{-5}$ and $p = 1.29 \times 10^{-5}$ respectively; Fig. 7). Most importantly, the RMS_H and RMS_L values were highly correlated ($r^2 \geq 0.96$ for both length and range) with the slopes and intercept not significantly different from 1 and 0 respectively (Table 1). The mean coefficient of variation of the repeated measurement was 6.1%, for the eight species. There was no apparent effect of a specific rig on RMS values, with a Chi square goodness of fit test indicating that none of the five rigs was more or less likely to have high RMS values ($\chi^2_{0.05[4]}; p = 0.52$).

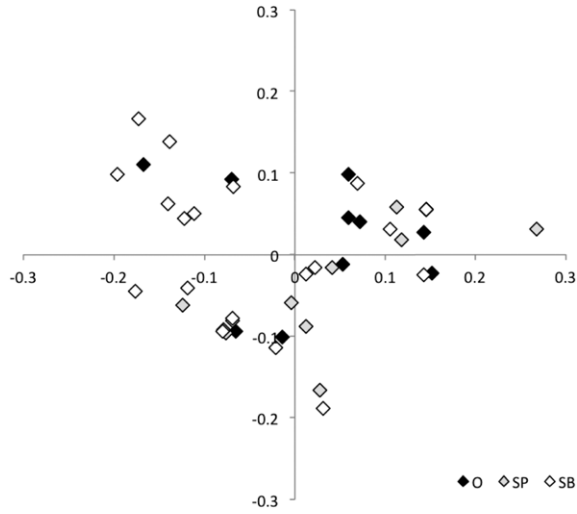


Fig. 5. Canonical analysis of principal coordinate analysis and multidimensional scaling of site similarity, by location (Ocean, South Passage, and Sandy Bay). Canonical axes separated fish assemblages present at different locations.

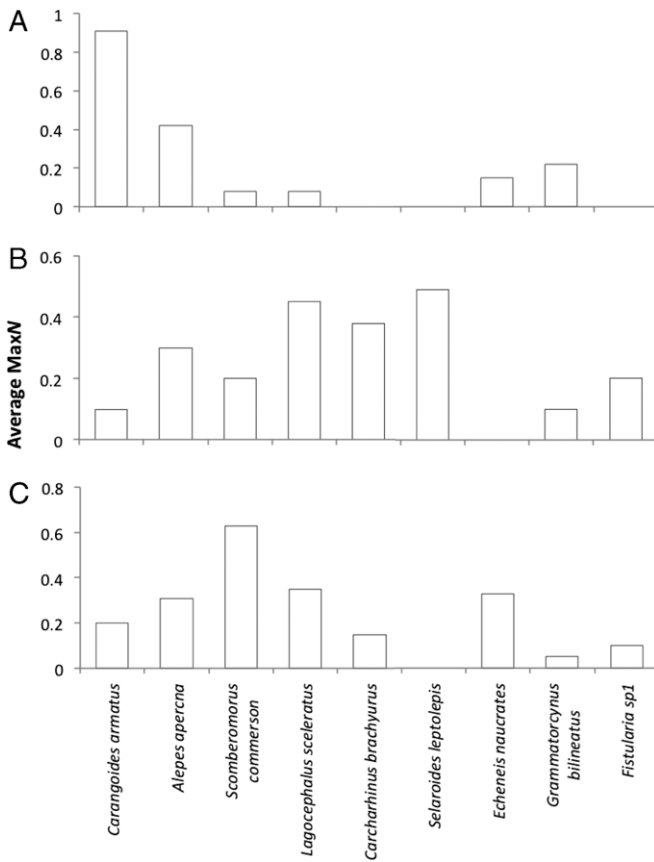


Fig. 6. Average species abundance (Max N), at Ocean location (A), South Passage (B), and Sandy Bay (C).

Table 1

Regression statistics for RMS_H vs RMS_L values for Length and Range based on $n = 8$ species, where MSE is the model standard error and we report the intercept (B_0) and slope (B_1) with their associated standard errors (SE) and lower and upper confidence limits (CL_L – CL_U).

Model metric	Length	Range
p	2.44E–05	1.29E–05
R^2	0.96	0.97
MSE	0.115	0.059
B_0 (SE)	0.029 (0.23)	–0.15 (0.28)
CL_L – CL_U	0.54–0.6	–0.84–0.54
B_1 (SE)	1.038 (0.089)	1.07 (0.08)
CL_L – CL_U	0.82–1.26	0.87–1.27

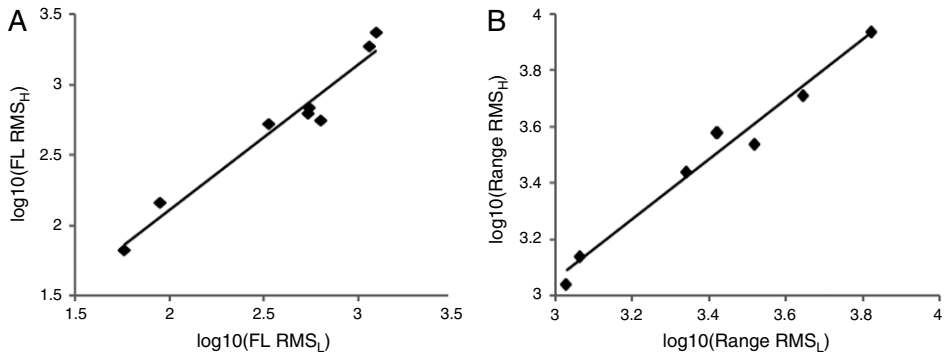


Fig. 7. Regressions showing relationship between mean values of high RMS lengths (A) and ranges (B) for fish species.

4. Discussion

The aims of this study were to demonstrate the capacity of mid-water baited camera surveys to describe pelagic fish assemblages and distinguish associations, nominally as a function of location. In order to maximise analytical power in future studies, we aimed to conduct attractant trials, and characterise the use of abundance proxies such as Max N and time of Max N . Here, our proof-of-concept study demonstrates that mid-water cameras can be used to distinguish assemblages and produce accurate measurements and range estimates.

4.1. Species accumulation and effect of location

The low rate of species accumulation across both number of deployments and deployment duration is a direct consequence of the low overall densities of fish typical of the pelagic realm, compared with many demersal habitats (Misund, 1993). Similarly, the high variability in Max N is indicative of high spatio-temporal heterogeneity, and high packing density typically encountered in shoals and schools (Chapman and Clynick, 2006). It follows that sampling in the pelagic environment requires greater effort than in demersal habitats.

Due to the experimental design we cannot rule out the confounding effects of attractants and location (and potentially depth) and we thus refer to the effect of location as a nominal effect. We could not discern an effect of attractants within locations where both attractants were used, but this may also be an artefact of small sample sizes. Given that the locations differed quite markedly in their physical characteristics (depth, water movement), it is not unreasonable to assume that location may be influencing the assemblage composition, noting that location did not affect species richness or abundance, a possible consequence of the overall low numbers of species and individuals.

The high abundance of *Carangoides armatus* juveniles in the Ocean location is likely a function of positive thigmotaxis towards the camera structure (typical for many juveniles, see Chapman and Clynick, 2006) as the fish were not observed feeding on bait. Moreover, thigmotaxis would probably not influence the mid-water assemblage representation in the shallow waters (<10 m) of South Passage and Sandy Bay, where the influence of seabed rugosity competes with the rig structure as a source of shelter. Regardless of whether this is a true effect of location or not, this demonstrates that assemblages can be sufficiently sampled to distinguish differences at the compositional level.

Our decision to classify fish as either pelagic or demersal based upon the description from FishBase (Froese and Pauly, 2011) may not capture the subtle differences in habitat association amongst different species. Most hotspots of pelagic diversity occur in association with, or adjacent to, coral reefs, seamounts, shelf breaks or other prominent topographical features (Worm et al., 2003; Morato et al., 2010). Since species that preferentially associate with the seabed sometimes appeared at South Passage and Sandy Bay, we most likely lost power to distinguish fish assemblages by restricting our analysis to those species defined as ‘pelagic’, and the distinction in fish assemblage is probably conservative.

In contrast to Heagney et al. (2007)’s methodology, we did not cap deployment durations at 45 min, a value conventionally used in demersal baited remote underwater video systems studies (Watson et al., 2010). From our results, the highest rate of time of Max *N* occurred between 60 and 90 min (37%). Moreover, for the majority of our species (57%), Max *N* occurred after 45 min and some species were only ever observed after 90 min (*Strongylura leiura* and *Grammatocynus bicarinatus*). In contrast to the high fish densities observed on the seabed, animal numbers in the pelagic zone are low, and as such, it is not immediately clear that similar deployment duration in the pelagic would yield sufficient power to detect discrepancies in spatial or temporal patterns detected in the demersal over such time periods. It is likely that further sampling in Heagney’s study (Heagney et al., 2007), would have revealed higher abundances of more slow-swimming and/or rare species.

4.2. Stereo measurements

The analysis of high and low RMS value measurements suggested that length estimates derived from GoPros are sufficiently accurate to be used for calculating individual species biomass. Whilst the slope and intercepts were not significantly different from 1 and 0, the regression equation did indicate that on average, lengths and ranges associated with high RMS values were slightly overestimated compared to those derived from low RMS values. However, the high correlations we observed suggest that RMS_H values can be back-corrected to length and range estimates consistent with low RMS values. Length and range were not correlated with their associated RMS values, suggesting that high RMS values were independent of animal length and distance from the camera within the 8 m cutoff range.

Concerns have been raised with respect to the use of GoPro cameras for length based measurements as (1) they rely on image stabilisation algorithms that are unique to each individual camera and (2) GoPros utilise rolling shutters instead of global shutters, which can distort high speed objects, and thus lead to high RMS values (Liang et al., 2005). In the former case, we found no systematic variation in RMS values by rig. In the latter, a preliminary analysis of RMS values associated with objects of known lengths moving between 0.4 and 1.4 m/s showed no effect of speed (Meeuwig unpubl.), the slowest of which are equivalent to the cruising speeds calculated for reef sharks from stereo BRUVS (Ryan unpubl.). GoPro derived lengths are therefore unlikely to be problematic for most individuals apart from those moving at speeds more than three times that of a cruising shark. As analysts can also choose windows of relatively slow movement, this concern is likely largely irrelevant.

The mid-water rigs also allowed for similar rates of length measurements to be made in comparison to rates of demersal stereo-BRUVS. We estimated lengths for 42% of the observed individuals and 78% of the observed species. This level of efficiency is higher than that in a previous demersal fish survey in Shark Bay using stereo-BRUVS, where length estimates were made for 30% of observed individuals and 70% of the species (Liang et al., 2005). More generally, the mid-water rigs’ efficiency is similar or slightly less than that of a range of demersal stereo-BRUV surveys undertaken in tropical Western Australia, where on average 47% of individuals and 87% of species were measured (Meeuwig, unpubl. data). The ability to measure lengths for a proportion of individuals and species comparable to those

recorded by BRUV-based analyses in Shark Bay (Clough, 2010) is promising, and the proportion of species measured is likely to increase with increased sampling effort.

Although there are some minor differences at higher RMSs values, these are typically small and can likely be corrected with a reasonable level of confidence (see Letessier et al., 2013). However, there is less certainty where there are very few individuals observed for a given species, and where length can vary greatly (such as for large sharks, e.g. *Galeocerdo cuvier*), thus making assumptions about representative lengths problematic. Indeed, there should have been a poor match between RMS_L and RMS_H for larger animals given that different animals of potentially different lengths were measured. However, such variation was not observed generally. For the purpose of characterising fish assemblage size and biomass on an ecological level, minor differences in measurements constitute a relatively small source of error and should not prohibit the use of mid-water rigs. However, fine scale demographic analyses where precise information on length structure is required are not recommended until a clearer picture of the bias source and extent can be determined.

4.3. Limitations of the method

The confounding effect of a bait plume and the varying catchment area of a camera system based on bait mean that in the absence of some current proxy, areal estimates of abundance and biomass are problematic. To overcome this restriction, Heagney et al. (2007) and Priede and Merrett (1996) standardised Max N and time of first arrival values respectively by current-derived areal estimates, thus providing some information on areal density. For our analyses, we assumed that any localised current influence was consistent across our sampling location and estimate on relative measures of abundance. However, the inclusion of small current metres could address this concern.

Studies have demonstrated that demersal baited cameras yield comparatively higher power in distinguishing predator communities, but have lower resolution in detecting cryptic and small species (e.g. from the families Pomacentridae, Labridae, Scaridae) when compared with diver operated video transects and underwater visual surveys (UVC) (Langlois et al., 2010; Watson et al., 2010). Since pelagic species diversity is relatively low compared with that in the demersal environment, and dominated by secondary and tertiary consumers—and since diver-based methods are largely unfeasible in the mid-water, the need to resolve differences with small fish is relatively minimal.

We did find that our capacity to generate stereo metrics was species specific, with certain species consistently maintaining a distance to the cameras too high for adequate stereo-measurements (>8 m, for example species like *Gnathanodon speciosus*). This may constrain biomass assessments for consistently shy species, as accurate lengths are required to estimate biomass. Further studies should attempt to derive error budgets to enable the use of high RMS values measurements incurred by measuring shy and distant fish.

The current reliance on bait also represents a limitation. The method is non-destructive in terms of the assemblages of interest, thus being of use within no-take marine reserves. However, it still relies on the use of non-negligible amounts of bait. Pilchards and bait squid are of relatively low trophic level and are considered preferable for use as bait compared to high trophic level species. However, low trophic status has not stopped overexploitation in several occurrences and the use of bait should be carefully considered (Dickey-Collas et al., 2010). Development of alternative attractant methods will allow the minimisation of use of bait, further reducing the ecological impact of sampling.

4.4. Recommendations

We did not detect any clear indication of a species accumulation asymptote. As such, we recommend that sampling effort be derived from the slope of the species accumulation curve, following the recommendations of Thompson et al. (2003). We recommend a minimum of 3 h recording duration per deployment in order to incorporate the arrival of slow-moving and late-arriving species such as *Grammatorcynus bicarinatus* and *Strongylura leiura*. As the majority of baited camera systems deployed around Australia and New Zealand use pilchards (Dorman et al., 2012), we recommend using thoroughly homogenised pilchard slurry as bait, contained inside a perforated PVC containers, so as

to sustain continuous dispersal of scent throughout the 3 h and allow for standardisation by bait type subject to such time as artificial attractants are reliably available.

4.5. Conclusion

To date, few studies have applied baited camera techniques to the investigation of mid-water fauna (Kubodera et al., 2007), and fewer still on mid-water (~10 m) fish (Heagney et al., 2007). We have designed a simple and low-cost wildlife sampling and monitoring system that can be rapidly deployed from a range of small and large vessels. Mid-water cameras can be utilised in conjunction with conventional study techniques for pelagic species such as acoustic and satellite tagging, active acoustics, and CPUE-based fishery surveys, and can generate information relevant to biological conservation and fishery management.

Implementing pelagic MPAs relies to some degree on the identification and inclusion of representative pelagic communities (Game et al., 2009). The spatial management of the open ocean must necessarily consider scales much larger than those explored here, and requires sampling across seabed depths where moorings are no longer practical. We explored this by deploying a few drifting rigs in parallel with the sampling regime presented in this study but the results were not further analysed, due to low replication. We envisage further studies that would explore differences in species accumulation rates across deployment duration and sampling number between drifting and moored rigs.

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References

- Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinate: a useful method of constrained ordination for ecology. *Ecology* 84 (2), 511–525.
- Bailey, D.M., King, N.J., Priede, I.G., 2007. Cameras and carcasses: historical and current methods for using artificial food falls to study deep-water animals. *Mar. Ecol. Prog. Ser.* 350, 179–191.
- Bertrand, A., Josse, E., 2000. Acoustic estimation of longline tuna abundance. *ICES J. Mar. Sci.* 57 (4), 919–926.
- Block, B.A., Jonsen, I.D., Jorgensen, S.J., Winship, A.J., Shaffer, S.A., Bograd, S.J., Hazen, E.L., Foley, D.G., Breed, G.A., Harrison, A.-L., Ganong, J.E., Swithenbank, A., Castleton, M., Dewar, H., Mate, B.R., Shillinger, G.L., Schaefer, K.M., Benson, S.R., Weise, M.J., Henry, R.W., Costa, D.P., 2011. Tracking apex marine predator movements in a dynamic ocean. *Nature* 475 (7354), 86–90.
- Block, B.A., Teo, S.L.H., Walli, A., Boustany, A., Stokesbury, M.J.W., Farwell, C.J., Weng, K.C., Dewar, H., Williams, T.D., 2005. Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* 434 (7037), 1121–1127.
- Brill, R.W., Block, B.A., Boggs, C.H., Bigelow, K.A., Freund, E.V., Marcinek, D.J., 1999. Horizontal movements and depth distribution of large adult yellowfin tuna (*Thunnus albacares*) near the Hawaiian Islands, recorded using ultrasonic telemetry: implications for the physiological ecology of pelagic fishes. *Mar. Biol.* 133, 395–408.
- Brooks, E.J., Sloman, K.A., Sims, D.W., Danylchuk, A.J., 2011. Validating the use of baited remote underwater video surveys for assessing the diversity, distribution and abundance of sharks in the Bahamas. *Endang. Species Res.* 13 (3), 231–243.
- Cappo, M., Speare, P., De'ath, G., 2004. Comparison of baited remote underwater video stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-reefal areas of the Great Barrier Reef Marine Park. *J. Exp. Marine Biol. Ecol.* 302 (2), 123–152.
- Chapman, M.G., Clynick, B.G., 2006. Experiments testing the use of waste material in estuaries as habitat for subtidal organisms. *J. Exp. Marine Biol. Ecol.* 338, 164–178.
- Clarke, K.R., Gorley, R.N., 2006. *Primer v6: User Manual/Tutorial*.
- Clarke, S.C., McAllister, M.K., Milner-Gulland, E.J., Kirkwood, G.P., Michielsens, C.G.J., Agnew, D.J., Pikitch, E.K., Nakano, H., Shivji, M.S., 2006. Global estimates of shark catches using trade records from commercial markets. *Ecol. Lett.* 9 (10), 1115–1126.
- Clough, J., 2010. The structure of demersal fish assemblages of South Passage and Blind Strait, Shark Bay, University of Western Australia, School of Animal Biology.

- Collette, B.B., Carpenter, K.E., Polidoro, B.A., Juan-Jorda, M.J., Boustany, A., Die, D.J., Elfes, C., Fox, W., Graves, J., Harrison, L.R., McManus, R., Minto-Vera, C.V., Nelson, R., Restrepo, V., Schratwieser, J., Sun, C.-L., Amorim, A., Brick Peres, M., Canales, C., Cardenas, G., Chang, S.-K., Chiang, W.-C., De Oliveira Leite, N., Harwell, H., Lessa, R., Fredou, F.L., Oxenford, H.A., Serra, R., Shao, K.T., Sumaila, U.R., Wang, S.-P., Watson, R., Yáñez, E., 2011. High value and long life—double jeopardy for tunas and billfishes. *Science* 333 (6040), 291–292.
- Cullis-Suzuki, S., Pauly, D., 2010. Failing the high seas: a global evaluation of regional fisheries management organizations. *Mar. Policy* 34 (5), 1036–1042.
- Dagorn, L., Holland, K.N., Filmlalter, J., 2010. Are drifting FADs essential for testing the ecological trap hypothesis? *Fish. Res.* 106 (1), 60–63.
- Dickey-Collas, M., Nash, R.D.M., Brunel, T., van Damme, C.J.G., Marshall, C.T., Payne, M.R., Corten, A., Geffen, A.J., Peck, M.A., Hatfield, E.M.C., Hintzen, N.T., Enberg, K., Kell, L.T., Simmonds, E.J., 2010. Lessons learned from stock collapse and recovery of North Sea herring: a review.
- Doray, M., Petitgas, P., Josse, E., Reynal, L., 2009. The influence of the environment on the variability of monthly tuna biomass around a moored, fish-aggregating device. *ICES J. Mar. Sci.* 66 (6), 1410–1416.
- Dorman, S.R., Harvey, E.S., Newman, S.J., 2012. Bait effects in sampling coral reef fish assemblages with stereo-BRUVs. *PLoS One* 7 (7), e41538.
- Dulvy, N.K., Baum, J.K., Clarke, S., Compagno, L.J.V., Cortés, E., Domingo, A., Fordham, S., Fowler, S., Francis, M.P., Gibson, C., Martínez, J., Musick, J.A., Soldo, A., Stevens, J.D., Valenti, S., 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Cons.: Mar. Freshwater Ecosyst.* 18 (5), 459–482.
- Fernandes, P.G., Brierley, A.S., Simmonds, E.J., Millard, N.W., McPhail, S.D., Armstrong, F., Stevenson, P., Squires, M., 2000. Fish do not avoid survey vessels. *Nature* 404 (6773), 35–36.
- Froese, R., Pauly, D., 2011. FishBase. World Wide Web Electronic Publication.
- Game, E.T., Grantham, H.S., Hobday, A.J., Pressey, R.L., Lombard, A.T., Beckley, L.E., Gjerde, K., Bustamante, R., Possingham, H.P., Richardson, A.J., 2009. Pelagic protected areas: the missing dimension in ocean conservation. *Trends Ecol. Evol.* 24 (7), 360–369.
- Girard, C., Benhamou, S., Dagorn, L., 2004. FAD: fish aggregating device or fish attracting device? A new analysis of yellowfin tuna movements around floating objects. *Anim. Behav.* 67 (2), 319–326.
- Graves, J.E., 1996. Conservation genetic of fishes in the pelagic marine realm. *Cons. Genet.*
- Hampton, J., Sibert, J.R., Kleiber, P., Maunder, M.N., Harley, S.J., 2005. Fisheries: decline of Pacific tuna populations exaggerated? *Nature* 434 (7037), E1–E2.
- Harvey, E.S., Shortis, M., 1996. A system for stereo-video measurement of sub-tidal organisms. *Mar. Technol. Soc. J.* 29, 10–22.
- Heagney, E.C., Lynch, T.P., Babcock, R.C., Suthers, I.M., 2007. Pelagic fish assemblages assessed using mid-water baited video: standardising fish counts using bait plume size. *Mar. Ecol. Prog. Ser.* 350, 255–266.
- Hilborn, R., Walters, C.J., 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. *Rev. Fish Biol. Fish.* 2 (2), 570.
- Hyndman, R.J., Koehler, A.B., 2006. Another look at forecast accuracy. *Int. J. Forecast.* 22, 679–688.
- Isaac, J.D., Schwarzlose, R.A., 1975. Active animals of the deep sea-floor. *Sci. Am.* 233, 85–91.
- Jackson, J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Eerlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293 (5530), 629–637.
- Josse, E., Bertrand, A., Dagorn, L., 1999. An acoustic approach to study tuna aggregated around fish aggregating devices in French Polynesia: methods and validation. *Aquatic Living Res.* 12 (5), 303–313.
- Kaplan, D.M., Chassot, E., Gruss, A., Fonteneau, A., 2010. Pelagic MPAs: the devil is in the details. *Trends Ecol. Evol.* 25 (2), 62–63. Author reply 63–64.
- Koldewey, H.J., Curnick, D., Harding, S., Harrison, L.R., Gollock, M., 2010. Potential benefits to fisheries and biodiversity of the Chagos Archipelago/British Indian Ocean Territory as a no-take marine reserve. *Mar. Pollut. Bull.* 60 (11), 1906–1915.
- Kubodera, T., Koyama, Y., Mori, K., 2007. Observations of wild hunting behaviour and bioluminescence of a large deep-sea, eight-armed squid, *Taningia danae*. *Proc. R. Soc. B: Biol. Sci.* 274 (1613), 1029–1034.
- Langlois, T.J., Fitzpatrick, B.R., Fairclough, D.V., Wakefield, C.B., Hesp, S.A., Mclean, D.L., Harvey, E.S., Meeuwig, J.J., 2012. Similarities between line fishing and baited stereo-video estimations of length-frequency: novel application of Kernel density estimates. *PLoS One* 7 (11), e45973.
- Langlois, T., Harvey, E.S., Fitzpatrick, B., Meeuwig, J., Shedrawi, G., Watson, D.L., 2010. Cost-efficient sampling of fish assemblages: comparison of baited video stations and diver video transects. *Aquatic Biol.* 9 (2), 155–168.
- Langlois, T., Radford, B., Van Niel, K., Meeuwig, J.J., Pearce, A., Rousseaux, C., Kendrick, G.A., Harvey, E.S., 2012. Consistent abundance distributions of marine fishes in an old, climatically buffered, infertile seascape. *J. Global Ecol. Biogeogr.* 21 (9), 886–897.
- Letessier, T.B., Kawaguchi, S., King, R., Meeuwig, J.J., Harcourt, R., Cox, M.J., 2013. A robust and economical underwater stereo video system to observe Antarctic Krill (*Euphausia superba*). *Open J. Mar. Sci.* (3), 148–153.
- Liang, C.K., Peng, Y.C., Chen, H., 2005. Rolling shutter distortion correction. *Proc Spie.*
- MacKenzie, B.R., Mosegaard, H., Rosenberg, A.A., 2009. Impending collapse of bluefin tuna in the northeast Atlantic and Mediterranean. *Cons. Lett.* 2 (1), 26–35.
- MacLennan, D., 1992. Fisheries Acoustics.
- McLean, D., Harvey, E., Fairclough, D., Newman, S., 2010. Large decline in the abundance of a targeted tropical letrhinid in areas open and closed to fishing. *Mar. Ecol. Prog. Ser.* 418, 189–199.
- McLean, D.L., Harvey, E.S., Meeuwig, J.J., 2011. Declines in the abundance of coral trout (*Plectropomus leopardus*) in areas closed to fishing at the Houtman Abrolhos Islands, Western Australia. *J. Exp. Marine Biol. Ecol.* 406 (1), 71–78.
- Misund, O.A., 1993. Dynamics of moving masses: variability in packing density, shape, and size among herring, sprat, and saithe schools. *ICES J. Mar. Sci.* 50, 145–160.
- Morato, T., Hoyle, S.D., Allain, V., Nicol, S.J., 2010. Seamounts are hotspots of pelagic biodiversity in the open ocean. *Proc. Natl. Acad. Sci. USA* 107 (21), 9707–9711.

- Moreno, G., Josse, E., Brehmer, P., Nøttestad, L., Us, C.D.B., 2007. Echotrace classification and spatial distribution of pelagic fish aggregations around drifting fish aggregating devices (DFAD). *Aquatic Living Res.* 20 (4), 343–356.
- Myers, R.A., Worm, B., 2003. Rapid worldwide depletion of predatory fish communities. *Nature* 423 (6937), 280–283.
- Newman, S.J., Skepper, C.L., Mitsopoulos, G.E.A., Wakefield, C.B., Meeuwig, J.J., Harvey, E.S., 2011. Assessment of the potential impacts of trap usage and ghost fishing on the Northern Demersal scalefish fishery. *Rev. Fish. Sci.* 19 (2), 74–84.
- Polacheck, T., 2006. Tuna longline catch rates in the Indian Ocean: did industrial fishing result in a 90% rapid decline in the abundance of large predatory species? *Mar. Policy* 30 (5), 470–482.
- Priede, I.G., Merrett, N.R., 1996. Estimation of abundance of abyssal demersal fishes; a comparison of data from trawls and baited cameras. *J. Fish Biol.* 49 (sa), 207–216.
- Ramirez-Llodra, E., Brandt, A., Danovaro, R., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., Vecchione, M., 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7, 2361–2485.
- Schnute, J.T., Richards, L.J., 2001. Use and abuse of fishery models. *Can. J. Fish Aquatic Sci.* 58, 10–17.
- Sibert, J.R., Hampton, J., Kleiber, P., Maunder, M., 2006. Biomass, size, and trophic status of top predators in the Pacific Ocean. *Science* 314 (5806), 1773–1776.
- Sumaila, U.R., Zeller, D., Watson, R., Alder, J., Pauly, D., 2007. Potential costs and benefits of marine reserves in the high seas. *Mar. Ecol. Prog. Ser.* 345, 305–310.
- Thompson, G., Withers, P.J., Pianka, E.R., Thompson, S.A., 2003. Assessing biodiversity with species accumulation curves; inventories of small reptiles by pit-trapping in Western Australia. *Aust. Ecol.* 28, 361–383.
- Ugland, K., Gray, J., Ellingsen, K., 2003. The species-accumulation curve and estimation of species richness. *J. Anim. Ecol.* 72 (5), 888–897.
- Watson, D.L., Harvey, E.S., Fitzpatrick, B.M., Langlois, T., Shedrawi, G., 2010. Assessing reef fish assemblage structure: how do different stereo-video techniques compare? *Mar. Biol.* 157 (6), 1237–1250.
- Watson, D.L., Harvey, E.S., Kendrick, G.A., Nardi, K., Anderson, M.J., 2007. Protection from fishing alters the species composition of fish assemblages in a temperate-tropical transition zone. *Mar. Biol.* 152 (5), 1197–1206.
- Wirsing, A.J., Heithaus, M.R., Dill, L.M., 2007. Fear factor: do dugongs (*Dugong dugon*) trade food for safety from tiger sharks (*Galeocerdo cuvier*)? *Oecologia* 153 (4), 1031–1040.
- Worm, B., Lotze, H.K., Myers, R.A., 2003. Predator diversity hotspots in the blue ocean. *Proc. Natl. Acad. Sci. USA* 100 (17), 9884–9888.
- Zar, J.H., 1975. Biostatistical analysis. *J. Amer. Statist. Assoc.*