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Temporal and spatial trends in prey composition of wahoo *Acanthocybium solandri*: a diet analysis from the central North Pacific Ocean using visual and DNA bar-coding techniques

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RUNNING HEAD: *ACANTHOCYBIUM SOLANDRI* DIET

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A diet analysis was conducted on 444 wahoo *Acanthocybium solandri* caught in the central North Pacific Ocean longline fishery and a nearshore troll fishery surrounding the Hawaiian Islands from June to December 2014. In addition to traditional observational methods of stomach contents, a DNA bar-coding approach was integrated into the analysis by sequencing the *cytochrome c oxidase subunit 1* (*COI*) region of the mtDNA genome to taxonomically identify individual prey items that could not be classified visually to species. Prey diversity of *A. solandri* was higher offshore than from nearshore waters. Temporal shifts in prey composition were observed between summer and autumn. For nearshore-caught *A. solandri*, juvenile pre-settlement reef fish species from various families dominated the prey composition during the summer months, followed primarily by Carangidae in autumn months. Gempylidae, Echeneidae and Scombridae were dominant prey taxa from the offshore fishery. Molidae was a common prey family found in stomachs collected north-east of the Hawaiian Archipelago while tetraodontiform reef fishes, known to have extended pelagic stages, were prominent prey items south-west of the Hawaiian Islands. The diet composition of *A. solandri* was indicative of an adaptive feeder and thus revealed dominant geographic and seasonal abundances of certain taxa from various ecosystems in the marine environment. The addition of molecular bar-coding to the traditional visual method of prey identifications allowed for a more comprehensive range of the prey field of *A. solandri* to be identified and should be used as a standard component in future diet studies.

Key words: adaptive feeding; *CO1*; prey identification; trophic ecology.

INTRODUCTION

Detailed information concerning the connectedness of both target and non-target species on multiple trophic levels is fundamental to the goals of an ecosystem approach to fisheries management (Link & Browman, 2014). Trophic ecology is an integral branch of ecosystem studies, providing a key framework for understanding how the biological components in an ecosystem are connected (Belgrano *et al.*, 2005). The study of trophic relationships in marine ecosystems is integral to understanding the magnitude of anthropogenic impacts to marine organisms. In the context of fisheries, targeted fishing can often disproportionately affect trophic relationships, leading to changes in energy flow in marine ecosystems (Garrison & Link, 2000; Link & Garrison, 2002).

Trophic predator-prey interactions have traditionally been examined through visual surveying of stomach contents (Hyslop, 1980). Stomach content analyses provide a 'snapshot' of an individual's diet at a particular point in time and space. The main limitation of this time-consuming approach is that partially and easily digested prey items often obstruct high-resolution identifications of prey items (Baker *et al.*, 2014; Leray *et al.*, 2015). Further, the degree to which prey items can be identified is dependent on many factors, including: digestion rate of predators, handling time between capture and stomach processing,

temperature, morphology and bulk composition of prey, and the availability of appropriate taxonomic keys (Folkvord, 1993; Legler *et al.*, 2010; Carreon-Martinez *et al.*, 2011). Thus, the use of rapid, accurate methods to identify prey items at high taxonomic resolution is integral to understanding the complexities of trophic interactions in marine ecosystems.

DNA bar-coding is a powerful and efficient way of identifying organisms with high taxonomic resolution, and is becoming an integral tool to identify prey items from gut contents in a wide range of terrestrial and aquatic organisms (Pompanon *et al.*, 2012). The molecular bar-coding technique has been applied in tandem with the standard visual approach to stomach-content analyses for aquatic predators from freshwater to reef and pelagic ecosystems (Barnett *et al.*, 2010; Corse *et al*., 2010; Dunn *et al.*, 2010; Valdez-Moreno *et al.*, 2012). Hargrove *et al.* (2012) noted the advantage of using molecular methods in addition to direct microscopy of stomach contents to decrease the percentage of unidentifiable prey items of French grunt *Haemulon flavolineatum* (Desmarest 1823). Prey contents from the entire gut and faecal matter have also been successfully sequenced, highlighting the potential utility of bar-coding of even highly digested prey items (Deagle *et al.*, 2009; Leray *et al*., 2015).

Acanthocybium solandri is a major component of the pelagic fisheries in Hawaii. The Hawaiian longline fishery comprises the majority of the landings of pelagic fishes in Hawaii, primarily targeting bigeye tuna *Thunnus obesus* (Lowe 1839), yellowfin tuna *Thunnus albacares* (Bonnaterre 1788) and swordfish *Xiphias gladius* L. 1758. A plethora of incidental-caught species are also considered commercially significant including: billfishes (Istiophoridae), *A. solandri*, moonfish *Lampris* spp., sickle pomfret *Taractichthys steindachneri* (Doderlein 1883), mahimahi *Coryphaena hippurus* L. 1758 and escolar *Lepidocybium flavobrunneum* (Smith 1843). The longline fishery will hereafter be referred to

as the offshore fishery. The troll fishery is smaller in fleet size and commercial value relative to the offshore fishery and consists of a variety of fully recreational and subsistence to part- and full-time commercial fishers. This fishery usually targets *T. albacares*, skipjack tuna *Katsuwonus pelamis* L. 1758, billfishes, *C. hippurus* and *A. solandri*, and typically operates within 37 km (20 nautical miles) of the main Hawaiian Islands (Boggs & Ito, 1993). The troll fishery will hereafter be referred to as the nearshore fishery.

There has been a lack of detailed information on the diet composition of *A. solandri*, a popular game and commercial fish species, in the central North Pacific Ocean. *Acanthocybium solandri* usually spend > 97% of their time inhabiting the upper epipelagic zone in the warm mixed layer, *i.e.* the upper 50 – 100 m of the water column (Iversen & Yoshida, 1957; Sepulveda *et al.*, 2011) and are generally regarded as epipelagic predators (Zischke, 2012). Previous diet studies on *A. solandri* reported a wide variety of fishes and squids with vast regional differences in prey composition with substantial proportions of unidentified organisms (Iversen & Yoshida, 1957; Manooch & Hogarth, 1983; Franks *et al.*, 2008). Due to the extensive spatial ranges of migratory pelagic fishes like *A. solandri*, examining regional differences in foraging habits are integral to fully understanding the feeding ecology (Buckley & Miller, 1994; Young *et al.*, 1997). Further, since *A. solandri* are caught in both offshore and insular environments, the vast differences in the prey resources in these different ecosystems will influence the dietary composition and highlight the opportunistic or adaptive nature of most pelagic predators (Bertrand *et al.*, 2002).

The feeding habits of *A. solandri* were examined for individuals collected from the Hawaiian nearshore troll and offshore longline fisheries during the summer and autumn of 2014. To increase the taxonomic resolution of prey identifications, the *cytochrome c oxidase*

subunit 1 (*COI*) region of the mtDNA genome was sequenced for individual prey items that could not be identified to species level visually. The addition of molecular bar-coding to the traditional visual method of examining stomach contents was expected to decrease the amount of unidentifiable prey items in the analysis and provide a more comprehensive taxonomic characterization of the prey field of *A. solandri*.

MATERIALS AND METHODS

SAMPLE COLLECTION

Stomachs from *A. solandri* caught in the offshore fishery were collected by observers from the National Oceanic and Atmospheric Administration (NOAA) Pacific Islands Regional Office (PIRO) Observer Programme from June to December 2014. The observer programme operates within the Hawaiian longline vessel fleets and collects stomachs while the catch is being gutted and cleaned at sea. The domain of the fishery encompasses the tropical and subtropical central North Pacific Ocean from 4 to 33 °N and 140 to 168 °W (Fig. 1). Each sampling site consisted of one fishing event. One stomach per site was collected at the majority of the sites (*c.* 70% of all sites), with two or three stomachs per site collected at the remaining sites. Stomachs were immediately frozen following removal from *A. solandri*. Geographical position and capture date of each individual were recorded on board. Sex and fork length (L_F) were sporadically recorded and thus were not included in the analysis, however, *A. solandri* specimens were generally between 100 and 170 cm L_F.

Individuals caught on trolling vessels were collected at the United Fishing Agency fish auction. These specimens were often still intact upon arrival at the auction, and thus entrails were provided by willing seafood wholesalers. Location of capture was not recorded for

specimens collected in the nearshore fishery, but fishers in the troll fishery who sell their catches usually operate around the islands of Oahu and Molokai (dotted black box, Fig. 1). All stomachs were either processed immediately after retrieval, or kept frozen for future processing. The date when an *A. solandri* was at auction was considered the date when the stomach was collected, but note that *A. solandri* were caught 1 to 3 days before they arrived at the auction block.

STOMACH PROCESSING AND PREY IDENTIFICATIONS

Stomachs were thawed, dissected laterally, and evacuated of all stomach contents. From the stomach contents, prey items were first visually identified to the lowest taxonomic level using available taxonomic keys (primarily Carpenter & Niem, 1999*a*, *b*, 2001*a*, *b*; Randall, 2010) and weighed on a digital scale (precision of 0.01 g). Depending on the physical quality of the prey items, fork, standard or mantle lengths of prey items were also recorded. Pacific saury *Cololabis saira* (Brevoort 1856) was used as bait in the offshore fishery, and these carcasses were excluded from the analysis. *Hirudinella ventricosa* is a common stomach parasite in *A. solandri* and were counted and weighed, but not included in the diet analysis. It was assumed for the purposes of this study that artificial lures were exclusively used in the nearshore fishery to remove the ambiguity of erroneously describing bait items as actual prey items.

Pieces of muscle or mantle tissue from prey items that could not be identified from taxonomic keys were carefully excised and stored in salt-saturated 20% DMSO. Scalpels and forceps used to excise tissue were thoroughly rinsed with water followed by 70% isopropyl alcohol between tissue excisions to avoid DNA cross-contamination of prey samples. DNA was extracted *via* the hot sodium hydroxide and tris method (HotSHOT; Meeker *et al.*, 2007).

To evaluate the correspondence between visual and metabar-coding taxonomic identifications, tissue samples from 20 known prey items each from 20 *A. solandri* were sequenced. These 20 taxonomic control samples were all correctly matched between a visual species identification and a genetic species identification with > 97% nucleotide similarity from the corresponding Bar-code of Life Database sequence (BOLD; Ratnasingham & Hebert, 2007).

MOLECULAR BAR-CODING

For prey item tissue samples, the *COI* region of the mitochondrial genome was amplified using primers *FISH-BCL* (5' TCA ACY AAT CAY AAA GAT ATY GGC AC 3') and *FISH-BCH* (5' ACT TCY GGG TGR CCR AAR AAT CA 3') (Baldwin *et al.*, 2009). Each 20 µl reaction included: 8.6 µl nanopure H_2O, 10 µl MangoMix (2x; Bioline; www.bioline.com/), 0.2 µl of each primer (10 µM) and 1 µl DNA (5 – 50 ng μl^{-1}). The thermocycling regime was as follows: 94 ºC for 3 min, 30 cycles consisting of 94 ºC for 30 s, 55 ºC for 45 s and 72 ºC for 45 s, and then a final extension period of 72 ºC for 10 min.

Primer combination *mICOlintF* (5' GGW ACW GGW TGA ACW GTW TAY CCY CC 3'; Leray *et al.*, 2013) and *jgHCO2198* (5' TAI ACY TCI GGR TGI CCR AAR AAY CA 3'; Geller *et al.*, 2013) as recommended by Leray *et al.* (2013) were used for samples that could not be amplified by the Baldwin *et al.* (2009) primers to increase amplification rates of prey sequences. Each 20 µl reaction included: 8.5 µl nanopure H_2O, 10 µl MangoMix (2x; Bioline), 0.2 µl of each primer (10 µM), 0.15 µl bovine serum albumin (10 mg ml^{-1}) and 1 µl DNA (5 – 50 ng μl^{-1}). A 'touchdown' PCR thermocycling profile was implemented similar to Leray *et al.* (2013) to minimize the probability of non-specific amplifications.

The PCR product was run on a 1.5% agarose gel and amplification success was defined as a single intense band around 600 bp for the Baldwin *et al.* (2009) primers or 300 bp for the Leray *et al.* (2013) primer set. The post-PCR cleanup process consisted of 6 µl PCR product and 0.9 µl ExoSAP-It (Affymetrix; www.affymetrix.com/) heated to 37 ºC for 30 min and then 85 ºC for 15 min. All PCR product preparation was conducted within the ToBo Laboratory at the Hawaii Institute of Marine Biology, University of Hawaii. Cleaned PCR products were sent to the Advanced Studies in Genomic, Proteomics, and Bioinformatics Genomics Laboratory at the University of Hawaii for single-direction sequencing. Sequences were compared to the BOLD (Ratnasingham & Hebert, 2007) and GenBank (Benson *et al.,* 2014) databases to infer taxonomic identity using a threshold of e 97% nucleotide similarity to distinguish species-level identifications. All bar-coded prey identifications, their nucleotide similarities to the BOLD and GenBank (Benson *et al*., 2014) databases, and internal validation bar-codes are provided in the Supporting information (Table SI). Prey sequences were submitted to GenBank under the accession numbers XXXX – XXXX.

Comment [J1]: Author to add

DIET ANALYSES

Sampling effort was evaluated using a prey species accumulation curve produced separately for *A. solandri* caught from the nearshore and offshore fisheries. Estimates of asymptotic species richness with percentile confidence intervals were calculated using the 'Chao1984' function from the 'vegan' package (Oksanen *et al.*, 2013) in the R statistical software (R 3.1.2, R Development Core Team; www.r-project.org) based on methods by Chao (1984).

Multiple bulk indices were used to assess prey species dominance. Prey abundance (*N*) was recorded as count data, prey mass (*M*) was recorded as a continuous variable and prey

frequency (*F*) was recorded as presence or absence (1 or 0, respectively). Per cent mass (%*M*), per cent abundance (%*N*) and per cent frequency (%*F*) were calculated for the i^{th} prey taxon as follows: $\%M_i = 100\ (\sum_j M_{ij}\ )\ (\sum_i \sum_j M_{ij}\ )^{-1}$, $\%N_i = 100\ (\sum_j N_{ij}\ )\ (\sum_i \sum_j N_{ij}\ )^{-1}$ and $\%F_i = 100\ (\sum_j F_{ij}\ )\ (p)^{-1}$, where *i*: 1, 2, 3, …, *n*, that refers to the total number of prey species, and *j*: 1, 2, 3, …, *p*, that refers to the number of predators (*i.e.* the number of stomach samples; Hyslop, 1980).

Using the bulk measurement indices, an index of relative importance (I_{RI}; Pinkas *et al.*, 1971) for the i^{th} prey species was then calculated using the equation: $I_{RIi} = (\%N_i + \%M)(\%F_i)$. I_{RI} values were then standardized to equal 100% (%I_{RI}).

Bulk indices were initially calculated for unidentified fishes and cephalopods to evaluate the contribution of unidentified prey items to the prey composition. %I_{RI} for each prey taxa was then recalculated excluding unidentified fishes and cephalopods. Due to their ability to accumulate in stomachs for long periods of time (Ridoux 1994; Lick & Piatkowski 1998), cephalopod beaks were not included in this analysis, but were frequent in *A. solandri* stomachs.

Multivariate techniques were used to analys the diet composition. The response variable for the multivariate analyses was a prey matrix consisting of the average of the %*N* and %*M* of a prey taxon for each individual predator (Assis, 1996; Bizzaro *et al.*, 2009). A principal components analysis (PCA) was conducted to explore inter-predator variation in diet composition using the vegan package (Oksanen *et al.*, 2013) in the R statistical software (R 3.1.2, R Development Core Team). PCA bi-plots were used to demonstrate the degree of interspecific variability in prey composition of the host species among major axes of variation

(Crespin de Billy *et al.*, 2000). Spatial and temporal trends in the diet compositions were examined for *A. solandri* caught in both fisheries using a canonical correspondence analysis (CCA; ter Braak, 1986). Due to the lack of spatial information on *A. solandri* catch locations in the nearshore fishery, only temporal variability (sampling month) in the diet composition was explored. For the offshore fishery, sampling month and a south-west to north-east (SW-NE) gradient were used as explanatory variables to explore both spatial and temporal variability. The SW-NE gradient was created because most of the sites were aligned on this axis and its calculation is explained here. Briefly, a straight line was drawn through the Hawaiian archipelago by connecting the most extreme south-eastern and north-western land features, Hawaii Island (19.57 N; 155.50 W) and Kure Atoll (28.42 N; 178.33 W), respectively. For each site, the shortest distance to that line (orthogonal distance) was calculated. The SW-NE gradient was calculated as a continuous variable, with positive residuals corresponding to sites north-east of the Hawaiian archipelago and negative residuals corresponding to sites south-west of the Hawaiian archipelago. A permutation analysis of variance (PERMANOVA; Anderson, 2001; McArdle & Anderson, 2001) was used to provide hypothesis tests for the significance of sampling month and spatial index to the diet compositions for the two fisheries.

RESULTS

STOMACH COLLECTION

A total of 444 *A. solandri* stomachs were collected from both fisheries, with 211 stomachs from the nearshore fishery and 233 stomachs from the offshore fishery (Table I). Stomach collection by month was higher during the summer months than in the autumn

months for both fisheries, reflecting the seasonal availability of *A. solandri*. As a result, samples from October, November and December were pooled into a single category (OND). The proportion of empty stomachs was slightly higher for the stomachs collected from the nearshore fishery (36%) than that from the offshore fishery (25%). For the following analyses, only results from 310 stomachs are included (135 from the nearshore fishery and 175 from the offshore fishery) with prey items present during dissection.

PREY SPECIES RICHNESS

Molecular bar-coding of prey items greatly increased the observed prey species richness for both fisheries. Fifty-three prey species were identified from *A. solandri* collected from the offshore fishery, of which 40 species were exclusively identified using the molecular approach. Thirty-nine prey species were identified from *A. solandri* collected from the nearshore fishery, of which 26 species were exclusively identified using the molecular approach.

Seventy-three fish and cephalopod species in total were identified from 399 prey sequences. Of the prey sequences, 215 sequences matched sequences in the BOLD database with > 97% similarity. The remaining 184 sequences matched known sequences in the GenBank database, with 153 sequences > 97% nucleotide similarity. The remaining 31 from those 184 sequences had nucleotide similarities < 97% and thus were classified as unidentified prey items.

Thirty-nine prey species were observed in the diet of *A. solandri* collected in the nearshore fishery. The prey species accumulation curve [Fig. 2(a)] suggested that the observed species richness approached asymptotic prey species richness (95% C.I.: 40, 62), suggesting that an adequate number of samples were collected to describe the taxonomic

breadth of the diet composition. The observed prey species richness of 53 species for *A. solandri* collected from the offshore fishery was considerably lower than the estimated prey species richness of 88 [95% C.I.: 65, 155; Fig. 2(b)].

DIET COMPOSITION OF NEARSHORE *A. SOLANDRI*

Carangidae [*e.g. Decapterus macarellus* (Cuvier 1833)], Scombridae (*e.g. K. pelamis*), and Priacanthidae [*e.g.* presumably pelagic stages of juvenile *Heteropriacanthus cruentatus* (Lacépède 1801)] were the most frequent prey items found in the nearshore-caught *A. solandri*. Juvenile (presumably pelagic-staged) reef fishes from various families (*e.g.* Chaetodontidae, Monacanthidae and Dactylopteridae) were also numerically abundant, but less frequent. Juvenile reef prey species were between 20-50 mm standard length (L_S). The largest prey items were *K. pelamis* (*c.* 400 mm L_S) and *D. macarellus* (200-300 mm L_S) and, similar to many prey > *c.* 200 mm, were usually present in two or three pieces. Two species, *H. cruentatus* and *D. macarellus* dominated the prey composition, accounting for over half the %IRI (Table II). Other lesser-observed taxa included: *Auxis rochei* (Risso 1810), *Promethichthys prometheus* (Cuvier 1832), and juvenile pelagic-stage reef fish species *Dactyloptena orientalis* (Cuvier 1832), *Chaetodon kleinii* Bloch 1790, and *Pervagor spilosoma* (Lay & Bennett 1839). Cannibalism occurred in low proportions relative to the main prey species.

Carangidae, Scombridae, and Priacanthidae were dominant prey families of *A. solandri* collected from the nearshore fishery. The first three principal components explained 68% of the total variance [Fig. 3(a,b)]. The scores of the stomachs on the bi-plots revealed a variety of foraging habits. For example, stomachs with scores placed near the arrow ends of the three dominant prey families in the PCA bi-plots [Fig. 3(a)] would primarily contain that

prey family. Stomachs with scores placed near the origin were feeding on rare prey items, or were generalist feeders consuming a mixture of the three dominant prey items (Crespin de Billy *et al.*, 2000). The first two PCA axes captured the variation characterized by the three most dominant prey items. The third PCA axis highlighted numerically abundant, but less frequent juvenile reef fishes.

Month of capture explained 11% of the total variation in the prey composition of wahoo from the nearshore fishery (PERMANOVA: $F_{4,160} = 4.16$, $P < 0.001$). The first two CCA axes explained 72% of the constrained variation. CCA axis one was primarily a temporal gradient, separating samples from the early summer from those in the autumn months [Fig. 4(a)]. Carangidae was strongly associated with CCA axis one and was situated near the later summer and autumn months. Other numerically abundant reef fish families were also associated with CCA axis one, in proximity to the centroids of the early summer months (*e.g.* Dactylopteridae, Monacanthidae and Chaetodontidae).

DIET COMPOSITION OF OFFSHORE *A. SOLANDRI*

The %IRI scores of the stomachs from the offshore fishery showed a varied array of feeding habits similar to *A. solandri* collected from the nearshore fishery (Table III). Juvenile snake mackerel *Gempylus serpens* Cuvier 1829 dominated the diet of the offshore-caught *A. solandri* in frequency, abundance, and mass. *Gempylus serpens* were usually in an advanced stage of digestion and present in multiple pieces due to its long body shape (*c.* 300 mm L_S). Echeneidae (*i.e. Remora brachyptera* (Lowe 1839), 40-80 mm L_S) and Molidae (*Ranzania laevis* (Pennant 1776), *c.* 150 mm L_S) were present in 26 and 21% of the stomachs, respectively, and also were major components of the diet. Similar to the diets of *A. solandri*

from the nearshore fishery, cannibalism occurred in low proportions relative to the main prey species.

Gempylidae, Echeneidae, and Molidae were dominant prey items from the PCA biplot of the offshore fishery [Fig. 3(c)]. The first three principal component axes explained 56% of the variance. The third PCA axis captured the variation associated with less observed prey families, *e.g.* Bramidae (*Brama* spp.), Scombridae (*Thunnus* spp. and *K. pelamis*), and Balistidae (*Melichthys* spp.) [Fig. 3(d)].

Significant temporal and spatial differences in prey composition were observed in the diets of offshore *A. solandri* (PERMANOVA: $F_{4,160} = 1.751$, $P < 0.05$ for month of capture; $F_{1,160} = 6.78$, $P < 0.001$ for SW-NE gradient). Month of capture and position along the SW-NE gradient explained 5% of the total variation in prey composition. The first two CCA axes explained 68% of the constrained variation. The first CCA axis was primarily a spatial axis separating sites north-east and south-west of the Hawaiian archipelago [Fig. 4(b)]. Molidae was strongly associated with the SW-NE gradient, usually observed in the stomachs of *A. solandri* collected north-east of the Hawaiian archipelago [Fig. 5(e)]. In contrast, juvenile pelagic-stage reef-associated balistids were mostly observed in stomachs of *A. solandri* collected south-west of the Hawaiian archipelago [Fig. 5(f)]. Common prey families, Gempylidae, Scombridae and Echeneidae [Fig. 5(b)-(d)], were present throughout the sampling domain [Fig. 5(a)]. Temporal variation was characterized by inter-month differences in the prey composition. The three dominant prey families (Gempylidae, Echeneidae, and Molidae) were consistently dominant prey items across the sampling months. Temporal variation was mostly driven by increases in importance of reef-associated tetraodontids in July and August and diodontids in the autumn months.

DISCUSSION

A.canthocybium solandri are an ideal predator for prey bar-coding because although prey items were often in an advanced state of digestion, prey items were large enough for tissue to be excised from single individuals for DNA extraction. The present work provides another example of molecular bar-coding as a viable way to assess the diets of many top marine predators (*e.g.* Dunn *et al.*, 2010; Côté *et al.*, 2013). DNA bar-coding provides a clear advantage over the visual methods that require enumerable body parts (*e.g.* vertebrae, fin rays) that are often partially or completely digested (Leray *et al.,* 2015). Bar-coding greatly enhanced the identification of cephalopods in this study, which were usually in a state of advanced digestion when recovered from *A. solandri* stomachs such that visual inspection of body parts for visual identification was severely limited. The increase in the species richness of the diet of *A. solandri* due to the bar-coding technique was similar to other bar-coding studies (*e.g.* Dunn *et al.,* 2010; Côté *et al.*, 2013).

Without the use of genetic techniques, unidentified fishes and squids would have been the majority of the bulk indices (*i.e.*, %N, %F, and %M) and thus a large portion of the stomach contents would have not been used in the diet analysis. The low contribution of unknown prey items in this study due to the implementation of the bar-coding technique increases confidence that the sample of the diet reported herein is representative of what *A. solandri* were consuming in the context of the sample design of this study. While the authors strongly advocate the use of molecular approaches to perform stomach content analyses, they suggest that these methods are used in concert with the traditional visual methods, not as a replacement for them.

There were stark differences in the prey compositions between *A. solandri* caught in the nearshore and offshore environments. The major prey items were indicative of the environments where *A. solandri* were caught, with pelagic prey items consumed by offshore *A. solandri* and coastal and reef-associated prey items consumed by nearshore *A. solandri* (Fig. 3). The offshore-nearshore gradient in diet composition seen in *A. solandri* is apparent for other pelagic fishes, for example albacore *Thunnus alalunga* (Bonnaterre 1788) (Goñi *et al.,* 2011). The increase in reef fish prey items observed for pelagics caught in the vicinity of reef environments has also been shown for other tunas (Bertrand *et al.*, 2002). The main prey items shared by *A. solandri* in both regions were other pelagic scombrids (*i.e. K, pelamis*) and various cephalopods.

Acanthocybium solandri in the nearshore environment were more similar to other insular-caught epipelagic predators (Brock, 1984; Buckley & Miller, 1994; Bertrand *et al.*, 2002). The dominance of juvenile reef fishes in the diet of the nearshore-caught *A. solandri* illustrates a clear trophic interaction between nearshore reef and pelagic ecosystems. The presence of reef fishes in the diet in the early summer months coincides with peak recruitment of Hawaiian juvenile reef fishes (Walsh, 1987). Fish recruitment often exhibits high interannual variability (Walsh, 1987) and thus different reef species could be observed if this study was done in a different sampling year. Many reef fishes observed in the diets of *A. solandri* have reportedly episodic periods of strong recruitment (*e.g. P. spilosoma* and *Priacanthus meeki* Jenkins 1903; Hobson & Chess, 1996; Stimson, 2005). Previous diet studies on nearshore-caught tunas and billfishes have also noted the importance of a diverse group of reef fishes (*e.g.* Tetraodontiformes, Acanthuridae, Priacanthidae, Chaetodontidae) to their diets (*e.g.* Brock, 1984; Buckley & Miller, 1994). *Acanthocybium solandri* are

seasonally available in the nearshore fishery during spring and summer, possibly concurrent with an insular *A. solandri* spawning period (Uchiyama & Boggs, 2006). These large schools of juvenile reef fishes returning to the reefs to settle would be an easily acquirable source of food for *A. solandri* and other pelagic predators in the nearshore environment during the summer. The juvenile stages of reef fishes thus provide an important link in the marine food web as seasonal sources of easily acquired prey.

There was a clear temporal trend in the diet composition for *A. solandri* collected from the nearshore fishery from a mixed diet that consisted of various families of reef fishes and scombrids in the summer to a diet dominated by *D. macarellus* in late summer and autumn [Fig. 4(a)]. Scombrids are common prey items for apex predators including *A. solandri* (Iversen & Yoshida, 1957; Franks *et al.*, 2008) marlins (Abitia-Cardenas *et al.*, 1997; Abitia-Cardenas *et al.*, 1999; Moteki *et al.*, 2001) and other scombrids (Buckley & Miller, 1994). Iversen & Yoshida (1957) conducted a diet study on *A. solandri* in the equatorial Pacific Ocean and reported *D. macarellus, K. pelamis*, and various squids as major components of the diet as did this study, but a noticeable dearth of reef-associated fishes. However, their study did not sample individuals during the summer period and thus it is unknown whether reef fishes were also major prey constituents of the diet. The dietary switch to *D. macarellus* in the late summer to early autumn may correspond with a decrease in juvenile reef fishes in the coastal environment as they settle into their reef habitats. *Decapterus macarellus* are common coastal forage fishes predated by many piscivores including seabirds (Brown, 1975; Harrison *et al.*, 1983), sharks (Torres-Rojas *et al.,* 2010; Moreno-Sánchez *et al.,* 2012) , and scombrids (Buckley & Miller, 1994; Fofandi *et al.* 2012).

The diet composition of *A. solandri* collected from the offshore fishery consisted of prey spanning a diverse spatial range from reef, coastal, epipelagic, to mesopelagic ecosystems. Many of the prey families reported for *A. solandri* in this study were common for *A. solandri* in other areas. Carangidae, Scombridae, and various squids are common prey taxa common in *A. solandri* diet studies in the Gulf of Mexico (Franks *et al.*, 2008), Indian (Malone *et al.*, 2011) and Atlantic (Vaske *et al*. 2003; Rudershausen *et al.*, 2010) oceans, and the Galapagos Marine Reserve (Baque-Menoscal *et al.*, 2012). Exocoetids were the only commonly reported prey taxa that were not observed in this study, however *A. solandri* caught in the equatorial Pacific just south of the sampling domain in this study (Iversen & Yoshida, 1957) and in the western central Pacific (Allain, 2003) also were not consuming exocoetids. *Acanthocybium solandri* in the North Atlantic Ocean were also not feeding on exocoetids, but selective on scombrids (*Auxis* spp.; Rudershausen *et al.*, 2010). These studies in concurrence with the present study, reveal the vast regional differences in pelagic predator foraging habits.

The only consistency in these *A. solandri* diet studies, including this study, is that crustaceans were not major prey items. *Acanthocybium solandri* do not usually form large schools (Iversen & Yoshida, 1957), so their preference towards fishes and cephalopods may be a form of niche differentiation among other schooling predators, such as scombrids that chase large schools of crustaceans and other zooplankton in the water column (Buckley & Miller, 1994; Olson & Galván-Magaña, 2002).

There were significant spatio-temporal trends in diet composition of *A. solandri* collected from the offshore fishery. Month of capture had a significant effect on the diet composition. The change in diet composition over the sampling period highlights the variability of juvenile reef fish recruitment patterns with emphasis on families in the Order

Tetraodontiformes especially in July and August. Tetraodontiformes are known to have extended juvenile stages in the pelagic zone (Leis, 1978; Leis & Moyer, 1985). Thus, their presence in the diet of *A. solandri* collected offshore may not show clear seasonal patterns. However, their presence in stomachs caught west of the archipelago may be indicative of a westward flow from the Hawaiian archipelago predicated by westward-progressing mesoscale eddies and/or westward transport from the North Equatorial Current (Qiu *et al*., 1997). Coral reefs of Johnston Atoll and the Hawaiian Archipelago flank these south-western sites and thus this area may have more of a reef signature than sites north-east of the Hawaiian Islands. Juvenile *R. laevis* (Molidae) were generally concentrated at sites north-east of the Hawaiian Archipelago [Fig. 5(e)]. Molidae can be common prey items in the central North Pacific, as observed with *G. serpens* (Choy *et al.*, 2013), *T. albacares* (Fitch, 1950), and various seabirds (Harrison *et al.*, 1983). These organisms could be following the Transition Zone Chlorophyll Front (TZCF) that is positioned just north of the sampling domain (Polovina *et al.*, 2001). The TZCF is a dynamic oceanographic feature where certain marine organisms such as tunas and turtles congregate to forage (Polovina *et al.*, 2009). Very little is known of the ecology of *R. laevis* and so it is unknown whether the apparent congregation of *R. laevis* north-east of the Hawaiian archipelago is due to characteristics associated with spawning and/or its juvenile life history.

Gempylus serpens was the most ubiquitous prey item among *A. solandri* collected from the offshore fishery. It is often caught as non-commercial by-catch in the offshore fishery and occupies the same depth range as *A. solandri* (Nakano *et al.*, 1997). Predated *G. serpens* were usually in an advanced stage of digestion and thus it was difficult to record prey length, but based on body width, it is plausible that *A. solandri* were consuming smaller

juvenile *G. serpens* (*c.* 30 cm) that occupy shallower depths during the daytime. *Gempylus serpens* is a common prey item in various seabirds (Harrison *et al.*, 1983), indicating that they inhabit the epipelagic zone during the daytime. Catch rates of pelagic fishes in the offshore fishery over a 16 year period (1996 – 2011) indicated a fishery-induced change in the catch composition, with increased catch rates of mid-trophic level fishes such as *G. serpens*, *L. flavobrunneum* and lancetfish *Alepisaurus ferox* Lowe1833 (Polovina *et al.*, 2009; Woodworth-Jefcoats *et al.*, 2013). A decrease in top-level predators due to fishing pressure and a concurrent increase in mid-trophic by-catch fish species is concurrent with juvenile G. *serpens* being a presumably abundant prey source in the epipelagic zone.

It should be noted that due to the fisheries-dependent nature of the sample collections, the size range of the *A. solandri* represents the size range of the *A. solandri* caught in the fishery. The lower bound of the size range corresponds to less than a year of growth, with most of the *A. solandri* surveyed in this project between 0.5 and 3 yr (Zischke *et al.*, 2013). Thus, conclusions on diet composition from this study are characteristic of adult *A. solandri* vulnerable to the fishery. Further, due to the relatively short period of sample collection (< 1 yr), *A. solandri* during the winter-early spring period were not sampled. Due to the relative temporal stability of the diets of offshore *A. solandri* in this study, it can be hypothesized that the diet of offshore *A. solandri* would be relatively similar if sampled in the non-sampled months. They are unavailable in the nearshore fishery during the late-autumn - winter months and are assumed to be in the offshore environment during this period. The summer of 2014 was an anomalously successful recruitment year in the main Hawaiian Islands for various reef fish families (R. Humphreys, Jr., NOAA, pers. comm.), and interannual variation in the types of reef fishes observed in the diet is quite probable. Nevertheless, the prey dominance of reef

fishes is common to pelagic predators roaming nearshore insular areas (Brock, 1984; Buckley & Miller, 1994; Bertrand *et al.,* 2002). Additional sampling of nearshore-caught *A. solandri* in future years would be predicted to yield similar conclusions with regard to the importance of reef fishes in the diet. *Acanthocybium solandri* generally feed on a wide variety of fishes and squids from nearshore reef to epipelagic to mesopelagic environments and are thus an important trophic linkage among these radically different ecosystems. They were somewhat opportunistic in terms of their fish prey, but there was evidence of feeding selectivity towards a few dominant groups in the water column. Size was also an important characteristic of prey items, as most fish prey items were juveniles with the possible exception of scombrids. The foraging patterns of *A. solandri* in the nearshore coastal area were possibly influenced by local reef recruitment dynamics, and future studies should analyse the link between nearshore pelagic predators and reef recruitment. Distinct spatial patterns in prey occurrence were observed for offshore-caught *A. solandri*, highlighting the regional variability of *A. solandri* foraging patterns. Lastly, DNA bar-coding greatly aided in the identification of highly digested prey items, and should be considered alongside standard visual techniques in the protocols of future diet analyses.

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Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE S1: Sequence identifications, length, and percent similarities from the BOLD and GenBank (with ACCN) databases. Records in **bold** were prey items used for internal validation.

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TABLE I. *Acanthocybium solandri* stomach sample collections by fishery and month. Nearshore and Offshore refer to the Hawaiian troll and central North Pacific longline pelagic fisheries

Month	Nearshore	Offshore
June	58	51
July	54	56
August	35	42
September	44	54
OND	20	30
Total	211	233

OND, pooled samples from October, November and December.

TABLE II. Prey table for *Acanthocybium solandri* collected from the nearshore troll fishery showing bulk indices for the per cent mass (%*M*), per cent abundance (%*N*) and per cent frequency (%*F*) of prey items, along with per cent index of relative importance (%I_{RI}). Bulk indices for unidentified fishes and squids are shown, but were excluded from the bulk and compound indices calculations

Taxon	%*N*	%*M*	%*F*	%I_{RI}	VIS	MOL
Fishes						
Aulopiformes						
Alepisauridae						
Alepisaurus ferox	0.24	0.43	0.85	< 0.1		+
Perciformes						
Acanthuridae						
Acanthurus nigrofuscus	0.72	0.05	1.71	< 0.1		+
Acanthurus olivaceus	0.24	0.11	0.85	< 0.1		+
Naso lituratus	0.48	0.09	0.85	< 0.1		+
Naso unicornis	2.63	0.46	2.56	0.31		+
Bramidae						
Brama orcini	1.2	0.72	3.42	0.26		+
Brama sp.	0.24	0.28	0.85	< 0.1		+
Unidentified Bramidae	0.24	0.17	0.85	< 0.1	+	
Carangidae						
Caranx melampygus	1.44	0.63	1.71	0.14		+
Decapterus macarellus	7.42	29.66	19.66	28.88	+	+

Decapterus macrosoma	1.44	2.46	5.13	0.79		+
Decapterus maruadsi	0.48	0.72	1.71	< 0.1		+
Decapterus sp.	1.44	6.65	5.13	1.64	+	
Selar crumenophthalmus	0.48	0.48	1.71	< 0.1		+
Chaetodontidae						
Chaetodon kleinii	8.61	0.55	6.84	2.48		+
Chaetodon unimaculatus	1.2	0.18	0.85	< 0.1		+
Forcipiger flavissimus	0.48	0.09	1.71	< 0.1	+	+
Hemitaurichthys polylepis	0.24	0.07	0.85	< 0.1		+
Heniochus acuminatus	0.48	0.02	1.71	< 0.1		+
Unidentified Chaetodontidae	2.15	0.28	2.56	0.25	+	
Gempylidae						
Gempylus serpens	0.72	0.72	1.71	< 0.1		+
Promethichthys prometheus	5.5	3.49	5.13	1.83	+	+
Istiophoridae						
Makaira nigricans	0.24	0.22	0.85	< 0.1		+
Lutjanidae						
Lutjanus kasmira	0.24	0.03	0.85	< 0.1		+
Mullidae						
Mulloidichthys vanicolensis	0.48	0.16	0.85	< 0.1		+
Priacanthidae						
Heteropriacanthus cruentatus	23.68	16.86	28.21	45.31	+	+
Unidentified Priacanthidae	0.24	0.06	0.85	< 0.1	+	

Scombridae						
Acanthocybium solandri	4.07	2.26	6.84	1.72		+
Auxis rochei	1.67	11.83	5.13	2.74	+	+
Auxis thazard	0.24	0.24	0.85	< 0.1	+	+
Katsuwonus pelamis	4.55	7.66	12.82	6.20	+	+
Thunnus obesus	0.48	1.01	1.71	0.10		+
Unidentified Scombridae	0.72	2.27	2.56	0.30	+	
Siganidae						
Siganus spinus	0.72	0.06	1.71	< 0.1	+	
Scorpaeniformes						
Dactylopteridae						
Dactyloptena orientalis	8.85	1.15	7.69	3.05	+	+
Tetraodontiformes						
Balistidae						
Melichthys vidua	0.24	0.21	0.85	< 0.1	+	+
Sufflamen bursa	0.48	0.03	1.71	< 0.1		+
Sufflamen fraenatum	1.67	0.26	2.56	0.20		+
Molidae						
Ranzania laevis	0.48	1.19	1.71	0.11	+	+
Monacanthidae						
Cantherhines pardalis	1.2	0.17	0.85	< 0.1		+
Pervagor spilosoma	5.5	0.87	5.13	1.29	+	+
Unidentified Monacanthidae	3.83	0.65	5.98	1.06	+	

Tetraodontidae						
Lagocephalus lagocephalus	0.24	0.28	0.85	< 0.1	+	+
Unidentified Tetraodontidae	0.24	2.4	0.85	< 0.1	+	
Unidentified fishes	10.69	4.14	15.07			
Cephalopods						
Teuthida						
Ommastrephidae						
Notodarus hawaiiensis	1.67	1.77	3.42	0.47		+
Sthenoteuthis oualaniensis	0.24	0.1	0.85	< 0.1		+
Unidentified cephalopods	5.04	2.59	9.52			

+, indicates whether a prey taxon was identified visually (VIS) or using the molecular barcoding approach (MOL).

TABLE III. Prey table for *Acanthocybium solandri* collected from the offshore longline fishery showing bulk indices for the per cent mass (%*M*), per cent abundance (%*N*) and per cent frequency (%*F*) of prey items, along with per cent index of relative importance (%I_{RI}). Bulk indices for unidentified fishes and squids are shown, but were excluded from the bulk and compound indices calculations

Taxon	%*N*	%*M*	%*F*	%I_{RI}	VIS	MOL
Fishes						
Aulopiformes						
Alepisauridae						
Alepisaurus ferox	0.58	0.24	1.8	< 0.1		+
Beloniformes						
Exocoetidae						
Exocoetus monocirrhus	1.16	0.78	2.4	0.14	+	
Unidentified Exocoetidae	0.19	0.2	0.6	< 0.1	+	
Hemiramphidae						
Oxyporhamphus micropterus	1.16	0.77	1.8	< 0.1		+
Berciformes						
Anoplogastridae						
Anoplogaster cornuta	0.39	0.09	1.2	< 0.1		+
Berycidae						
Beryx splendens	0.19	0.14	0.6	< 0.1		+
Lampriformes						
Lophotidae						

Lophotus lacepede	0.19	0.86	0.6	< 0.1		+
Perciformes						
Bramidae						
Brama dussumieri	0.77	0.16	1.8	< 0.1		+
Brama orcini	4.82	1.76	10.78	2.11		+
Brama sp.	0.19	0.07	0.6	< 0.1		+
Pteraclis aesticola	0.58	0.23	1.8	< 0.1		+
Taractichthys steindachneri	1.35	1.63	4.19	0.37		+
Carangidae						
Decapterus macarellus	0.39	0.16	1.2	< 0.1		+
Decapterus macrosoma	0.19	0.2	0.6	< 0.1		+
Naucrates ductor	0.19	0.01	0.6	< 0.1		+
Chaetodontidae						
Forcipiger flavissimus	0.39	0.08	0.6	< 0.1	+	
Forcipiger longirostris	0.39	0.08	0.6	< 0.1	+	
Hemitaurichthys thompsoni	0.19	0.21	0.6	< 0.1		+
Heniochus acuminatus	0.19	0	0.6	< 0.1		+
Chiasmodontidae						
Dysalotus alcocki	2.12	0.35	6.59	0.48	+	+
Echeneidae						
Remora brachyptera	14.84	16.89	26.35	24.90	+	+
Remora sp.	1.73	1.52	2.99	0.29	+	
Gempylidae						

Gempylus serpens	19.27	12.45	41.92	39.60	+	+
Lepidocybium flavobrunneum	3.08	0.6	2.99	0.33	+	+
Nealotus tripes	2.12	0.89	4.79	0.43	+	+
Ruvettus pretiosus	0.58	0.1	0.6	< 0.1		+
Nomeidae						
Cubiceps baxteri	0.39	0.08	1.2	< 0.1		+
Cubiceps paradoxus	0.19	0.03	0.6	< 0.1		+
Cubiceps pauciradiatus	0.19	0.06	0.6	< 0.1		+
Psenes cyanophrys	1.35	0.49	2.4	0.13		+
Psenes maculatus	0.39	0.67	1.2	< 0.1		+
Scombridae						
Acanthocybium solandri	2.5	1.03	4.79	0.50		+
Auxis rochei	0.19	2.09	0.6	< 0.1	+	+
Katsuwonus pelamis	2.31	10.39	5.99	2.27	+	+
Thunnus alalunga	0.19	0.32	0.6	< 0.1		+
Thunnus obesus	0.58	4.78	1.8	0.29		+
Scombrolabrachidae						
Scombrolabrax heterolepis	0.19	0.01	0.6	< 0.1		+
Zanclidae						
Zanclus cornutus	0.19	0.01	0.6	< 0.1		+
Scorpaeniformes						
Dactylopteridae						
Dactyloptena orientalis	0.19	0.03	0.6	< 0.1	+	

Syngnathiformes						
Fistulariidae						
Fistularia petimba	0.19	0.03	0.6	< 0.1		+
Tetraodontiformes						
Balistidae						
Melichthys niger	6.94	4.59	7.19	2.47	+	+
Melichthys vidua	0.39	0.38	1.2	< 0.1		+
Sufflamen fraenatum	0.19	0.03	0.6	< 0.1		+
Unidentified Balistidae	0.19	0.24	0.6	< 0.1	+	
Diodontidae						
Chilomycterus reticulatus	0.19	0.1	0.6	< 0.1		+
Diodon sp.	0.19	0.21	0.6	< 0.1	+	
Diodon hystrix	0.19	0.1	0.6	< 0.1		+
Unidentified Diodontidae	1.73	3.94	4.19	0.71	+	
Molidae						
Masturus lanceolatus	0.58	0.18	1.2	< 0.1		+
Ranzania laevis	13.68	23.72	20.96	23.34	+	+
Tetraodontidae						
Lagocephalus lagocephalus	1.35	1.25	2.99	0.23		+
Sphoeroides pachygaster	0.19	0.47	0.6	< 0.1		+
Unidentified Tetraodontidae	3.85	2.19	1.2	0.22	+	
Unidentified fishes	6.81	1.84	12.2			

Cephalopods					
Octopoda					
Argonautidae					
Argonauta nodosa	1.54	0.74	3.59	0.24	+
Unidentified Octopoda	0.19	0.26	0.6	< 0.1	+
Oegopsida					
Ommastrephidae					
Hyaloteuthis pelagica	0.19	0.02	0.6	< 0.1	+
Teuthida					
Enoploteuthidae					
Enoploteuthis reticulata	0.19	0.01	0.6	< 0.1	+
Ommastrephidae					
Sthenoteuthis oualaniensis	1.54	0.81	4.79	0.34	+
Onychoteuthidae					
Onykia sp.	0.19	0.24	0.6	< 0.1	+
Unidentified cephalopods	2.96	1.53	9.88		

+, indicates whether a prey taxon was identified visually (VIS) or using the molecular barcoding approach (MOL).