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Article *in* Marine and Freshwater Research · November 2010

DOI: 10.1071/MF09261 · Source: OAI

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Diet and reproduction in the white-spotted eagle ray *Aetobatus narinari* from Queensland, Australia and the Penghu Islands, Taiwan

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Abstract. *Aetobatus narinari*, a circumglobal batoid, is subjected to increasing fishing pressures, especially throughout South-east Asia. However, its management and protection is complicated by the lack of relevant life history information. It appears to be a late-maturing, long-lived stingray with a size-at-maturity of ~130 and >150 cm in ventral disc width for males and females respectively. Like other myliobatids, *A. narinari* is a matrotrophic viviparous species exhibiting lipid histotrophy as indicated by trophonemata. Only the left ovary and uterus are functional. The presence of mature sperm in the testes, collecting ducts, epididymis and ductus deferens coincided with the estimated time of parturition and mating. Catches indicated an unbiased sex ratio. *Aetobatus narinari* is a hard-prey specialist that feeds mainly on gastropods, molluscs and hermit crabs (Diogenidae). Molluscs comprised numerically and gravimetrically the most important prey group (Index of Relative Importance (IRI): 85.9% in Australia, 99.9% in Taiwan) and were observed in 83.3% and 100% of stomachs containing food from Australia and Taiwan respectively. Minor dietary shifts from a gastropod–crustacean to a more gastropod–bivalve based diet occurred as body size increased. This study provides vital biological data for the effective management and conservation of *A. narinari*.

Additional keywords: Chondrichthyes, ecology, elasmobranch, life history, stingray.

Introduction

The white-spotted eagle ray, *Aetobatus narinari*, is the most common of three species within the genus. It is found circumglobally throughout temperate and tropical waters and inhabits mainly inshore areas and coral reef environments. While the species is considered 'near threatened' worldwide, including Australia and Taiwan, it has already been classified as 'vulnerable' on the IUCN Red List for Threatened Species (2006) in South-east Asia (Kyne *et al.* 2006). However, the lack of any detailed biological data on the species prevents an accurate assessment of the effects of current fishing pressures, and therefore adequate management and conservation measures. Lack of data also prohibits an accurate evaluation of *A. narinari*'s role as a potential threat to commercially farmed invertebrates, which has been shown to occur in other large schooling batoids (e.g. Smith and Merriner 1985; Peterson *et al.* 2001; Yamaguchi *et al.* 2005). Since 2001, over 10 000 longheaded eagle rays (*A. flagellum*) have been culled every year in a predator control program in Japan to protect commercially farmed bivalve stocks (Yamaguchi *et al.* 2005). Batoids may be particularly sensitive to harvests such as these owing to their life history characteristics (slow growth, late maturity, and low fecundity).

Apart from a few observations (Table 1), the reproductive biology of *A. narinari* has only been assessed by White and Dharmadi (2007), where a general assessment of batoid reproductive biology was undertaken in male specimens at various Indonesian fish markets. Size-at-maturity in females has never been assessed and previous accounts of the disc widths of mating or pregnant females have been largely estimations. Additionally, no detailed dietary information is currently available for *Aetobatus narinari*. The purpose of the current study was to provide some basic information on the food composition and reproductive biology of *A. narinari*. Two geographically distinct regions, Australia and Taiwan, were compared; these represent locations close to the northern and southern extent of the species geographical range. Finally, data were considered within the broader context of potential fishing pressures, conservation issues and ecosystem implications.

Material and methods

Collection

Aetobatus narinari were caught by local fishermen or collected from fish markets and by field sampling. Animals were caught

 10.1071/MF09261 1323-1650/10/111278

Table 1. Compilation of data on various aspects of reproduction in female *A. narinari*
–, data not available

Reference	Location	Size of female (cm)	Size at maturity (cm)	Litter size *n*	Litters per year *n*	Size of embryo (cm)	Time of year caught	Gestation period (days)
Mahon, Chua and Newman, unpub.	Underwater World Singapore	~160	–	1–2	3 in 2 years	48–45	–	180–188
Mahon, Chua and Newman, unpub.	Underwater World Singapore	~180	–	–	3 in 2 years	–	–	–
Mahon, Chua and Newman, unpub.	Sea World Texas	–	–	1–3	–	40–52	–	270–330
Mahon, Chua and Newman, unpub.	Okinawa Expo Aquarium	~140	–	1–2	–	50–59	–	331–377
White *et al.* 2007	Indonesia	–	–	–	–	<33.4	–	–
Homma *et al.* 2004	Micronesia	105–108.5	–	–	–	Max 40.6	–	~240
Homma *et al.* 2004	Micronesia	–	–	2	–	33.8 and 22.0*	February	–
Homma *et al.* 2004	Micronesia	–	–	1	–	–	July	–
Homma *et al.* 2004	Micronesia	–	–	1	–	–	September	–
Last and Compagno 1999	Central Pacific	–	214	4	–	17–36**	–	–
McEachran *et al.* 1999		–	–	–	–	18–36**	–	–
Last and Stevens 1994	Australia	–	–	1–4	–	>26**	–	–
Michael 1993		–		–	–	–	–	~365
Bigelow and Schroeder 1953	Atlantic	–	–	6–10	–	17–36**	–	–
Coles 1913	North Carolina, USA	~236	–	4	–	29*	Summer	–

*Embryo aborted prematurely.
**Estimated range.

Table 2. Catch composition and size distribution for the three collection sites

Region	Number of individuals (*n*)				Size (cm)		
	Total	Males	Females	Neonates	Range	Mean (males)	Mean (females)
Moreton Bay	73	36	37	11	47.7–122.9	63.7	65.1
Heron Island	5	1	4	0	112.0–192.2	167.6	144.9
Penghu Islands	41	25	16	0	57.2–160.7	106.9	79.9

by longline or gill-net. Fishing effort in Moreton Bay (27°15′S, 153°15′E) was constant throughout the year and most individuals were caught in the morning. A total of 78 *Aetobatus narinari* were collected from two sites in Australia, Moreton Bay (2005–2007, $n = 73$) and Heron Island (23°26′S, 151°55′E; February 2007, $n = 5$) and 41 individuals from the Penghu Islands, Taiwan (23°33′N, 119°35′E; April 2006) (Table 2). Of these, the reproductive status was assessed for 76 Australian and 35 Taiwanese individuals. Stomachs were collected from all individuals. There were no signs of regurgitation.

General dissection

Animals were weighed, measured and sexed. Stomachs and reproductive organs were removed from either freshly caught (<5 h) or thawed individuals and immediately stored in 10% formalin in dH_2O. Female organs included ovaries, oviducts and uteri. Reproductive status in females was assessed according to the presence of oocytes, ova and/or embryos and uterus size (including presence of trophonemata). Male organs examined included claspers and testes. Neonates were identified by their open yolk-sac scars. Stomach contents were sorted and assessed after preservation in formalin.

Ovaries were weighed to the nearest 0.1 g and uteri width measured at the widest point to the nearest 1 mm. In mature specimens, we recorded whether ovaries (both or single) and uteri were functional (based on the presence of oocytes, enlarged uteri and trophonemata). Stage 1 juvenile females had no oocytes and thin strap-like uteri. Stage 2 sub-adult females showed ovarian follicles undergoing vitellogenesis but had neither enlarged uteri nor trophonemata. Mature females (adults) possessed small, medium and/or large-yolked oocytes and had an enlarged and fully developed left uterus with trophonemata present, either without embryos and uterine eggs (stage 3), containing embryos and/or uterine eggs (stage 4) or showing signs of recent parturition (stage 5). The diameter of each oocyte was measured to the nearest 0.1 mm. The maximum oocyte diameter (MOD) was also recorded.

Maturity in males was based on the calcification status and length of claspers. Juvenile males had small, soft and flexible claspers (stage 1, non-calcified). Sub-adults had larger, harder and partially flexible claspers (stage 2, not fully calcified). Adults had very large and rigid claspers (stage 3, fully calcified). Calcification status was recorded for 55 males; whereas clasper length measurements were recorded for 52 males. C01 was defined as the outer clasper length, measured from the point where clasper and pelvic fin merge to the tip of the clasper and C02 as the inner clasper length, measured from the tip of clasper to the anterior end of the cloaca. Depending on calcification, individuals were assigned a binomial maturity status (immature, 0; mature, 1) and data binned into 5-cm size classes. The disc width at which 50% of males reach maturity (DW_{50}) was established using logistical regression analysis, i.e. FitLogistic (Hall 2001):

$$P_{DW} = P_{max}[1 + \exp(-\ln(19)(DW - DW_{50})/(DW_{95} - DW_{50}))]$$

where P_{DW} refers to the probability of an individual with disc width DW to be mature, DW_{50} and DW_{95} represent constants (Walker 2005) and P_{max} is 1 (as all animals were assumed to be able to reach maturity) (Marshall *et al.* 2007). Maximum likelihood estimates were calculated in SOLVER (Microsoft Excel) and probabilities for the occurrence of immature ($1 - P_{DW}$) (stages 1 and 2) and mature (P_{DW}) rays were determined. Median values (Fig. 1*b*) were derived from re-sampling data randomly with a bootstrap value of 100. The 95% confidence intervals were the 2.5 and 97.5 percentiles of all values predicted by the bootstrap analysis (Walker 1992; Marshall *et al.* 2007; White and Dharmadi 2007). Additionally, the relationship between clasper length (C_L) and disc width (DW) was described by a general logistic function (Hall 2001) as:

$$C_L = b + \{(a - b)/[1 + \exp(-\ln(19)(DW - DW_{50})/(DW_{95} - DW_{50}))]\}$$

where C_L is the estimated clasper length at disc width DW, a is the theoretical maximum and b the theoretical minimum length (mm) that can be attained by the claspers (Marshall *et al.* 2007; White and Dharmadi 2007).

Histology

The reproductive organs from the only freshly caught mature male were removed and fixed in 10% formalin in seawater. Following histological processing to paraffin wax, small tissue sections from the testis, epididymis and ductus deferens were removed, sectioned and stained with haematoxylin and eosin and Mallory's Trichome.

Stomach contents

Stomach contents were carefully rinsed to remove any debris and mucus. Prey items were sorted according to digestion state, from 1 (undigested, good condition) to 5 (digested and unidentifiable). Complete items (states 1 and 2) were counted and weighed (wet mass) to the nearest 0.001 g, whereas partially digested, but identifiable, material (state 3) was enumerated by

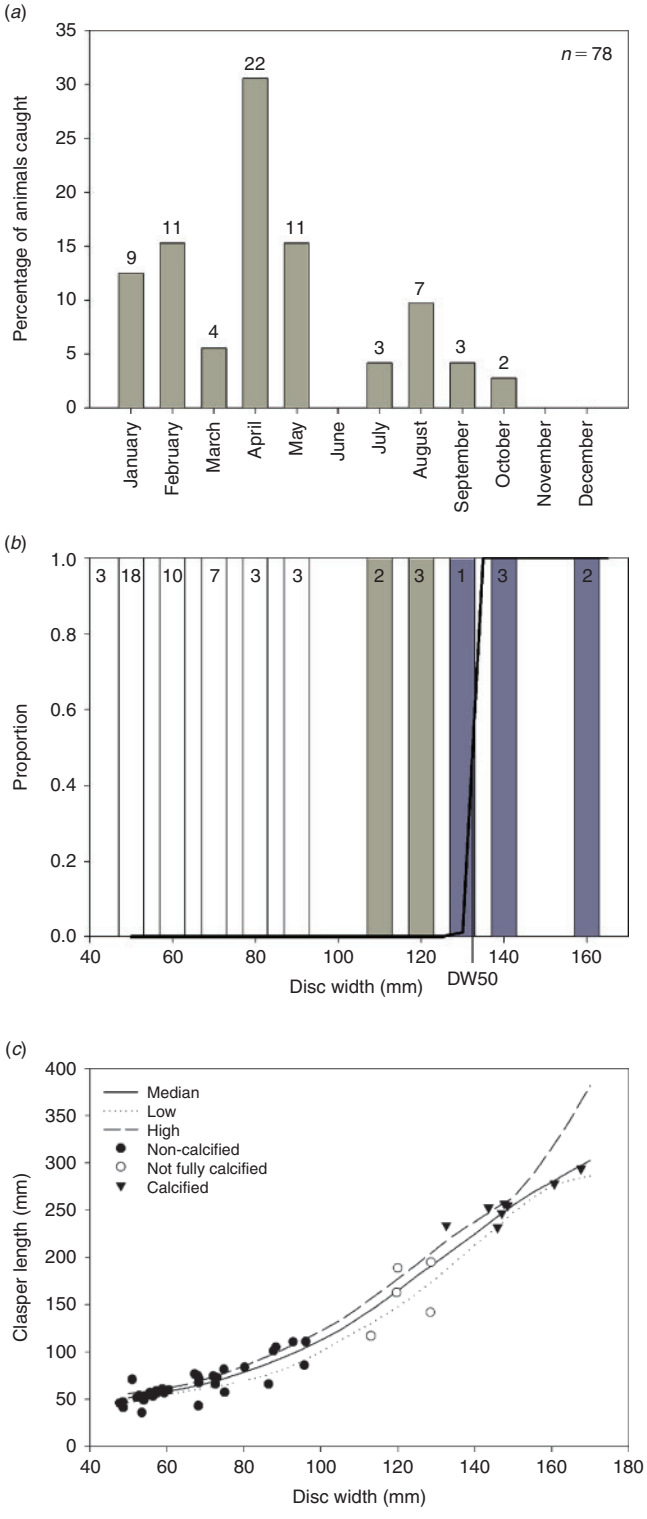

Fig. 1. (*a*) Comparison of monthly catch rates in Moreton Bay over a two-year period (July 2005 to July 2007); numbers above bars denote total *n* per month. (*b*) Proportion of immature (white bars), sub-adult (light grey bars) and mature (grey bars) males in Australia and Taiwan as a function of DW. A logistic curve was fitted to the data, with the arrow denoting DW_{50}. (*c*) Relationship between clasper length and disc width. A logistic curve including 95% confidence intervals was fitted to the data.

defining a unique part, e.g. the abdomen of a hermit crab, and weighing that part. Mass and numbers of state 3 items were then added to the mass and numbers of complete items (1 and 2) within the same prey group. State 4 items were identifiable, but could not be counted. Their mass was recorded and added to the mass obtained for prey items of the same species in states 1, 2 and 3. Unidentified objects (state 5) were weighed, but excluded from further analyses. Final numbers and masses for each prey group were recorded for each stomach. Hard parts, such as operculae or carapaces, were included if they were still attached to the animal. Prey species were identified to the lowest possible taxonomic level.

Analysis – Index of Relative Importance

Data were analysed using the Index of Relative Importance (IRI, Pinkas *et al.* 1971):

$$IRI = (N_c + M_c)F_0$$

where N_c is the numerical abundance of a prey item, M_c represents the mass of a prey item and F_0 describes the frequency of occurrence for that particular prey item. Percent IRI (%IRI) followed the approach of Cortés (1997, 1999). The percentage of mass and the numerical contribution of each prey group was calculated instead of its percent volumetric contribution, as mass provided a more reliable indicator of prey representation in the presence of many small items (Hyslop 1980). The %IRI was calculated for each phylum and class of prey species and separate IRI tables were constructed for *A. narinari* from Australian and Taiwanese waters. The importance of functional groups – such as crustaceans or molluscs – was assessed so that stomachs containing items that were not identifiable to lower taxonomic levels could be included in the analysis (White *et al.* 2004).

Statistical analysis

Data were tested for differences in the dietary composition of Australian males and females, for all males and females combined (Australia and Taiwan) and for potential dietary shifts between different size classes (Australia ventral disc width (DW): <65 cm, 65–90 cm, >90 cm; Combined: <60 cm, 60–79 cm, 80–110 cm, >110 cm) using the program PRIMER version 5 (Clarke and Gorley 2001). Regional differences between Australia and Taiwan were also assessed in PRIMER. Groups within Taiwan were not statistically compared as sample sizes were too small. Because IRI tables indicated that regional diets were similar, regional data were pooled and analysis repeated for the combined dataset.

All stomachs were characterised by an abundance of items from just one or two of the six prey groups. To assess the data in a statistically robust manner, individuals within a particular category (e.g. male, female or a specific size class) were randomly assigned to groups of four, and averages for their stomach content data were analysed (Linke *et al.* 2001; Platell and Potter 2001; White *et al.* 2004). Mean mass (g) contributions of each of the six individual prey categories (crustacean, gastropod, bivalve, polychaete, echinoderm and unidentified mollusc) were square-root transformed and similarity matrices constructed using the Bray–Curtis similarity coefficient.

Matrices were ordinated by non-metric multidimensional scaling (nMDS) and groups compared with a one-way Analysis of Similarity (ANOSIM) (Clarke and Gorley 2001). Similarity percentages (SIMPER) were calculated to reveal what prey species characterised the diet of individuals within a particular category, as well as which prey species were responsible for the greatest dietary differences between categories (Clarke 1993). A Chi-square test was performed to determine sex ratios of eagle ray catches in Australia and Taiwan.

Results

Sex ratios and size at birth

Sixty-eight percent of rays in Australia were caught during April–May and January–February (Fig. 1*a*). *Aetobatus narinari* caught in Taiwan and Heron Island were only collected during April and February respectively; monthly catches therefore could not be compared. Male-to-female ratios of eagle ray catches in Australia ($n = 78$) and Taiwan ($n = 41$) were 0.95 : 1 and 1 : 0.73 respectively, and showed no significant bias ($\chi^2_1 = 0.205$, $P = 0.651$, Australia; $\chi^2_1 = 0.684$, $P = 0.408$, Taiwan). Faint yolk-sac scars were recorded in all rays up to 92.8 cm DW in Australia and up to 96.1 cm DW in Taiwan. Open yolk-sac scars were recorded in 11 neonates that were caught in Moreton Bay either during May (45%), April (18%) or January (9%). No neonates were caught in Taiwan. Neonates (five females and six males) ranged in size between 48.6 cm and 57.0 cm. The smallest individual measured 47.7 cm, but did not have an open yolk-sac scar, which suggests that size at birth may show considerable variability, from below 47.7 cm to over 57.0 cm DW.

Female reproduction

Of the 56 females examined during this study, one was mature (stage 4, adult); six were classified as stage 2 and 49 were juveniles (stage 1). Only the left ovary appeared to be functional, in that it contained immature and/or yolky oocytes, whereas the right ovary, although of similar size, was not observed to contain follicles or oocytes. Oocytes were small and whitish or large and yellow (yolky), and were observed from individuals with a disc width of ≥106.1 cm ($n = 7$). Oocytes were extremely fragile, ruptured easily and, in general, most of the female reproductive system seemed to disintegrate rapidly after death. In two cases, neither ovarian size or mass nor oocyte number or oocyte size could therefore be determined. In juvenile females, right and left ovary masses were similar (Table 3) but in all stage 2 females for which reproductive systems could be collected in good condition ($n = 6$), the left ovary was considerably larger. Stage 2 females had 8–11 oocytes in their left ovary, with maximum oocyte diameters (MOD) of 1.5–2.0 cm. The single stage 4 female ovary contained 24 oocytes, most of which were very small (Table 3), although three were large (up to 4.5 cm in diameter) and yolky. The presence of mature oocytes and embryos in the same individual indicated that vitellogenesis proceeds in parallel with gestation. Ovulation may therefore occur shortly after parturition.

The stage 4 female, caught in February near Heron Island, aborted four live embryos that were estimated to measure ≥35 cm DW. Both of the uteri were enlarged, with the left uterus more than twice the size of the right uterus and lined with abundant trophonemata, secreting a whitish histotroph. No trophonemata lined the right uterus but the uterine walls were dark red, indicating a high degree of vascularisation. Neither the right nor the left uterus contained any uterine ova. The presence of trophonemata confirmed that *A. narinari* is an aplacental matrotroph (in addition to the yolk reserves, the fetal development is supplemented to various degrees by some other nutritious input from the mother, such as uterine secretions, i.e. histotroph (Musick and Ellis 2005)) that exhibits lipid histotrophy. Presently, size-at-maturity can only be estimated to occur at ≥150 cm DW. Two large females (145.6 cm and 152.5 cm DW) were classed as sub-adults, and the only pregnant female caught measured 192.2 cm DW.

Male reproduction

Of the 55 males assessed during this study, 41 were juveniles, five were sub-adults and eight were adults. There was no overlap in size ranges of juvenile *v.* sub-adult and sub-adult *v.* adult individuals. All males smaller than 96.1 cm in DW had non-calcified claspers, rays between 113.0 cm and 128.6 cm DW had partially calcified claspers, and the claspers of all males over 132.6 cm DW

Table 3. Maturity status of the eight largest females caught during this study
NR = not reported, NA = not applicable

Ventral DW	Stage	TM	Number of embryos	Size of embryos	Ovary size (g)		Number and size (cm) of oocytes in left ovary				MOD
					Left	Right	Large	Medium	Small	Extra small	
192.2	4	Yes[A]	4[B]	~35 cm	160	130	3 (4.1–4.5)	2 (2.6–2.7)	9 (1.5–1.8)	10 (<0.6)	4.5
152.2	2[C]	NR	0	NA	NR	NR	Oocytes present but all split: no measurements possible				
145.6	2	No	0	NA	73.1	55.5					
130.0	2	No	0	NA	60.32	47.77			10 (1.01.6)		1.6
122.9	2	No	0	NA	NR	NR	Oocytes present but all split: no measurements possible				
112.0	2	No	0	NA	58.13	30.3			2 (~1.0)	9 (0.4–0.6)	1.5
106.1	2	No	0	NA	NR	NR			3 (~1.5)	5 (0.4–0.6)	1.5
97.8	1	No	0	NA	16.43	15.25	No oocytes present				

[A]Only found within left uterus.
[B]Aborted during capture but viable.
[C]Not properly assessed (due to market conditions).

were fully calcified (Table 4). The theoretical size at which 50% of the population reach maturity (DW_{50}), based on clasper calcification, was 130.6 cm DW (Fig. 1*b*), with a DW_{95} of 131.1 cm. A weak sigmoidal relationship was observed between disc width and clasper length (C02). The DW_{50} based on clasper length was calculated to be 132.5 cm (Fig. 1*c*). Clasper length increased rapidly with increases in DW at the onset of maturity (Table 4). Both models therefore gave very similar values for DW_{50}.

Males had testes of similar size, each consisting of many lobes, in a 'compound' organisation typical of batoids (Pratt, 1988). Spermatocysts containing immature sperm (e.g. spermatogonia or spermatocytes) were found in the central regions of each lobe, close to the germinal zone, whereas more mature spermatocysts containing spermatozoa and bundles of sperm were found more peripherally (Fig. 2*a–d*). Mature sperm were also observed within the collecting efferent ductules (Fig. 2*e*) and sperm residue was found within the epididymis and the ductus deferens (Fig. 2*f*).

Stomach contents

Of the 78 Australian rays and 41 Taiwanese rays, 11.8% and 12.2% respectively had empty stomachs. One ray stomach from Australia and two from Taiwan contained only unidentifiable matter and another 17 from Taiwan leaked during transport; accordingly, these were excluded from further investigation. Stomach contents of 67 Australian and 18 Taiwanese rays were used in subsequent analyses. Australian rays contained a total of 5806 prey items with a combined mass of 1.037 kg (27.6 g were unidentified object matter and not included in the analysis). Taiwanese rays contained 856 items, weighing only 147.81 g. However, Taiwanese stomachs contained large amounts of digested matter, which was unidentifiable and not included in the analysis.

Stomachs from Australian individuals usually had only one or two prey species. Molluscs were by far the most important prey group (85.9% IRI) for *A. narinari*, both on a numerical (74.6%) and a gravimetric (78.0%) level (Table 5). Most of the molluscs, present in 83.3% of all stomachs, were gastropods (66.8% of total numbers, 56.7% of total mass), which mainly belonged to the family Trochidae (possibly *Austrocochlea*, *Heteropoma* and *Calliostoma*: J. Healy, pers. comm.) and to a lesser extent, to the family Cerithiidae (possibly *Cerithium*, *Rhinoclavis* or *Clypeomorus*: J. Healy, pers. comm.). Bivalves (none of which could be identified to a lower level), contributed little in terms of frequency of occurrence (9.1%) and numbers (7.0%) but still provided 21% of the overall consumed mass. The second most important prey group was crustaceans (14.1% IRI), which were present in 43.9% of all stomachs. Crustaceans contributed 22.0% to overall mass and 25.4% to overall numerical abundance and were almost exclusively (except two Ocypodidae) hermit crabs of the family Diogenidae. Polychaetes and echinoderms occurred in 3% and 1.5% of all stomachs, respectively, and contributed negligible amounts to overall numbers, mass and %IRI.

Stomach contents from Taiwanese individuals consisted almost exclusively of molluscs (99.9% IRI), which occurred in all stomachs (100%), and constituted 99.3% of overall numbers and 97.9% of overall mass of prey. Most molluscs were unidentified gastropods (74.6% of the overall mass) and bivalves (7.4%). The only other prey group was crustaceans (0.2% IRI), present in 10.5% of all stomachs but contributing little to overall numbers (0.7%) and mass (2.1%) of prey.

Statistical analysis of dietary differences

There were no significant differences between the diets of Australian male and female eagle rays (ANOSIM, Global $R = -0.052$, $P = 0.68$) and diets of males and females in general (Global $R = -0.002$, $P = 0.426$), so data were pooled in all further analyses. There was a small difference between diets of Australian *v.* Taiwan eagle rays (Global $R = 0.244$, $P = 0.04$); however, as Taiwanese samples were so few in number and could not be assessed independently of the Australian samples, analyses were performed for Australian samples alone and then repeated using pooled data from both regions.

Australian individuals were divided into three groups: small (<60 cm), medium (60–90 cm) and large (>90 cm) disc width. Significant differences were observed in the dietary compositions of these three groups (Global $R = 0.567$, $P = 0.003$; Fig. 3*a*, *c*). Small rays consumed mainly gastropods (52.8%) and crustaceans (42.5%) and to a much lesser extent bivalves

Table 4. Calcification status and clasper length (C01 & C02) for 52 males from Australia and Taiwan
DW gives the actual recorded disc width for each range. NC, non-calcified; NFC, not fully calcified; FC, fully calcified

Range	*n*	DW (cm)	Calcification level	C01 (mm)	C02 (mm)
40–49	3	48–49	NC	5.9–6.6	41.2–47.1
50–59	16	51–59	NC	6.0–8.6	36.0–71.0
60–69	6	60–68	NC	9.2–12.6	43.1–76.7
70–79	7	72–75	NC	5.0–10.5	57.4–81.5
80–89	4	80–88	NC	11.0–16.0	66.0–105.0
90–99	3	93–96	NC	18.0–22.0	86.0–111.0
100–109	0	–	–	–	–
110–119	2	113–119	NFC	23.0–35.0	117.0–163.0
120–129	3	120–129	NFC	24.0–61.0	142.0–195.0
130–139	1	133	FC	77.0	234.0
140–149	5	143–149	FC	80.0–95.0	232.0–257.0
150–159	0	–	–	–	–
160–169	2	160–167	FC	90.0–95.0	278.0–294.0

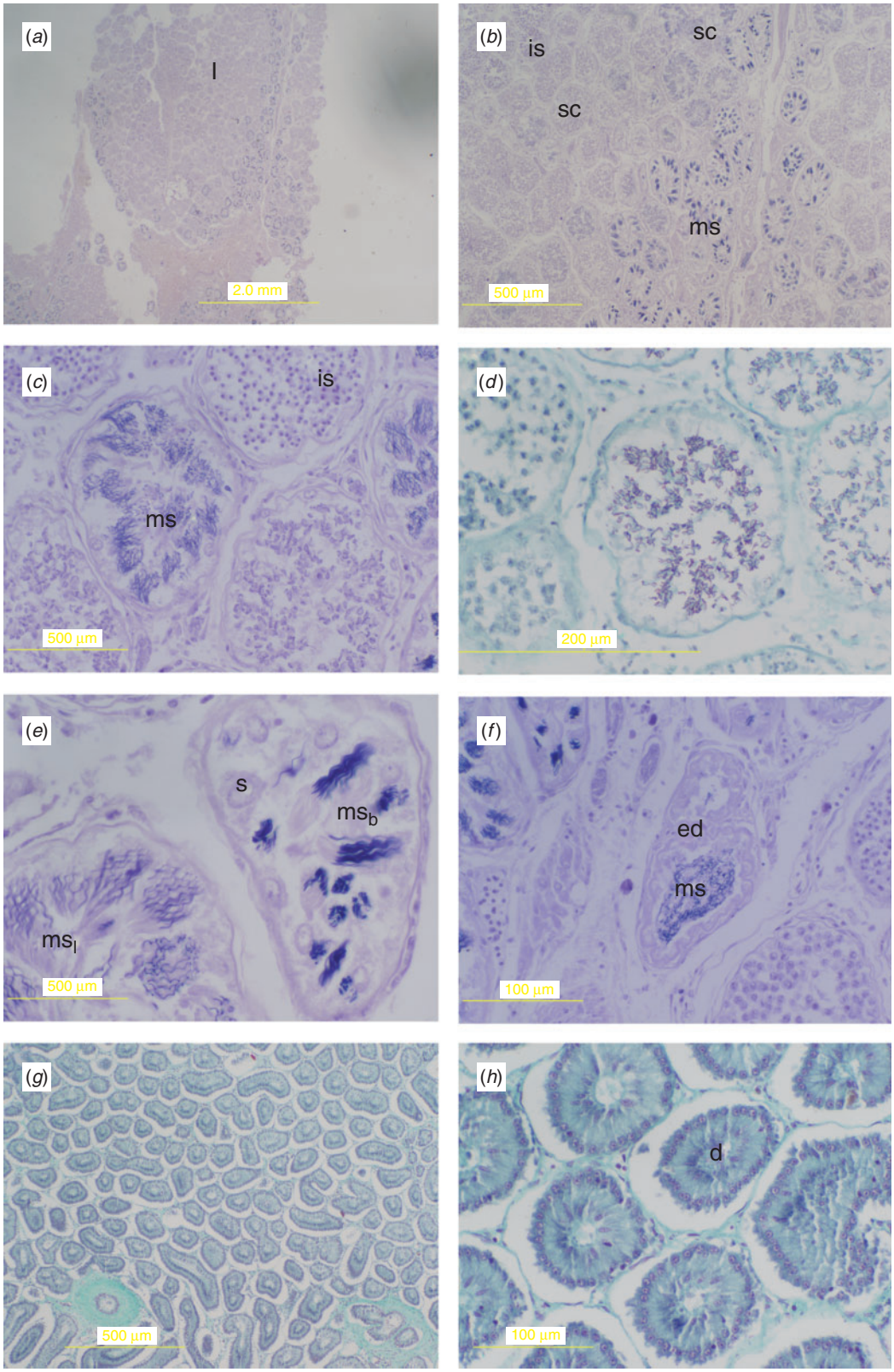

Fig. 2. (*a*) Testicular lobe in cross-section, showing spermatocysts with germ and Sertoli cells at various degrees of maturation. (*b*) More mature spermatocysts are located peripherally. (*c*, *d*) Spermatocysts with germ and Sertoli cells at various degrees of maturation. (*e*) Mature sperm arranged in loose (ms_l) and tightly packed bundles (ms_b). (*f*) Efferent ductules containing sperm and (*g*, *h*) epididymis with sperm residue. l, lobe; sc, spermatocysts; is, immature sperm; ms, mature sperm; ms_l, mature sperm (loose); ms_b, bundles of mature sperm; ed, efferent ductule; s, Sertoli cell; d, duct.

Table 5. Dietary composition of *Aetobatus narinari*, collected from Australia and from Taiwan

$\%F_0$ denotes the frequency with which a particular prey species occurred, $\%N_c$ the percentage contribution to numerical abundance and $\%M_c$ the percentage contribution to overall mass. %IRIs are shown for phyla and classes, chosen for comparison

Prey	$\%F_0$	$\%N_c$	$\%M_c$	%IRI	
				Phylum	Class
Collected from Australia					
Crustacea	43.9	25.4	22.0	14.1	–
Malacostraca	43.9	25.4	22.0	–	17.8
Anomura	–	–	–	–	–
Diogenidae	42.4	25.4	21.9	–	–
Brachyura	–	–	–	–	–
Ocypodidae	1.5	<0.1	0.1	<0.1	–
Annelida	3.0	<0.1	<0.1	–	–
Polychaeta	3.0	<0.1	0.1	–	<0.1
Echinodermata	1.5	<0.1	<0.1	<0.1	–
Ophiuroidea	1.5	<0.1	<0.1	–	–
Mollusca	83.3	74.6	78.0	85.9	–
Unknown	3.0	0.8	0.3	–	<0.1
Bivalvia	9.1	7.0	21.0	–	2.2
Gastropoda	75.8	66.8	56.7	–	80.0
Unknown	10.6	2.1	16.0	–	–
Trochidae	66.7	63.6	37.8	–	–
Cerithiidae	3.0	1.1	2.9	–	–
Collected from Taiwan					
Crustacea	10.5	0.70	2.12	0.15	–
Malacostraca	10.5	0.70	2.12	–	0.19
Anomura	10.5	0.70	2.12	–	–
Mollusca	100.0	99.30	97.88	99.85	–
Unknown	5.3	5.61	15.92	–	0.71
Bivalvia	10.5	3.27	7.40	–	0.71
Gastropoda	94.7	90.42	74.56	–	98.39

(4.1% by mass) (Fig. 3*a*). With increasing individual size, contributions of molluscs (gastropods and bivalves) increased to 71.3% (mass), whereas the proportion of crustaceans decreased to 20.6%. In the large size class, crustaceans only constituted a small amount of the diet (11.6%), whereas bivalves provided 36.3%. Gastropods were still the most abundant prey group, even in large rays (52.2%).

Australian and Taiwanese individuals were divided into four groups: small (<60 cm), medium (60–79 cm), large (80–110 cm) and extra large (>110 cm) disc widths. Significant differences were observed in the dietary compositions of these four groups (Global $R = 0.515$, $P = 0.001$; Fig. 3*b*, *d*). Combined groups (Fig. 3*b*) displayed similar trends to Australian groups (Fig. 3*a*). The contribution of crustaceans in the combined analysis decreased from 34.7% (mass) in small individuals to 0.6% in extra large individuals, whereas the contribution of bivalves increased from 7.6% in small individuals to 44.1% in extra large individuals. At least half of the food at any given time consisted of gastropods (50.0–73.4%).

Ordinations of the mean contribution to diet based on mass for each of the three (Fig. 3*c*) and four (Fig. 3*d*) size classes showed spatial clustering of groups. For Australian eagle rays (Fig. 3*c*), significant differences in dietary composition were observed between small and medium (ANOSIM, $P = 0.024$, $R = 0.357$) as well as between small and large size classes (ANOSIM, $P = 0.015$, $R = 0.952$), but not between medium and large size classes (ANOSIM, $P = 0.133$, $R = 0.429$). In the combined analysis (Fig. 3*d*), significant differences were observed between all size classes (ANOSIM, $0.01 \leq P \leq 0.02$, $R = 0.202$–1.0) except between small and large individuals, and between large and extra large individuals.

Discussion

General occurrence

Despite continuing efforts by commercial fishermen, few *A. narinari* were caught in Moreton Bay during winter. Decreases in catch rates coincided with a lower seasonal abundance of other elasmobranch species (S. Taylor, pers. comm.), possibly in response to lower water temperatures. Overall low catch rates in the second half of the year (June through December) could indicate that some individuals or groups reside in Moreton Bay year-round, whereas others only stay seasonally. Maximum catch rates during late summer and autumn (January to May) seemed to coincide with time of parturition, based on the presence of neonates only during these months (fishermen and Sea World employees (Gold Coast, Australia), pers. comm.).

On Heron Island, eagle rays usually occurred in groups at specific sites, where they were seen feeding together, but preferentially travelled in pairs or groups of three across the reef flats. In Moreton Bay, *A. narinari* was also usually observed and caught in groups. As eagle rays were only sampled for one month in Taiwan, no general observation regarding variations in the local catch composition could be made. However, local fishermen reported that eagle rays are usually caught throughout the entire year.

Female reproduction

The collection of large mature animals proved extremely difficult; additionally, bad preservation of specimens often resulted in disintegration of ovaries and testes. Owing to the low numbers of mature females, neither uterine fecundity, gonadosomatic nor hepatosomatic indices could be assessed. *Aetobatus narinari* has a similar reproductive mode to other members within the Myliobatidae. It is a matrotrophic viviparous species that bears few young, appears to mate soon after parturition (mature oocytes present during late gestation) and as suggested by previous studies, may have a gestation period of about one year (Table 1).

Only the left ovary and probably only the left uterus are functional in this species. Coles (1913), who examined several mature eagle rays, also found the left uterus in *A. narinari* to be much larger than the right and the only one to contain trophonemata (secreting the histotroph), indicating only left uterine functionality. A non-functional right uterus was observed in several related species, such as *Pteromylaeus bovina*, *Aetomylaeus nichofii* (Babel 1967) and *Rhinoptera bonasus* (Smith and Merriner 1986). However, in *Myliobatis californica*, *Myliobatis aquila* and *Urolophus paucimaculatus* both uteri were fully functional (Martin and Cailliet 1988; White and Potter 2005; Capapé *et al.* 2007). While uterine functionality varies even across species within the same family, all of the previously mentioned rays have only a left functional ovary. Unfortunately,

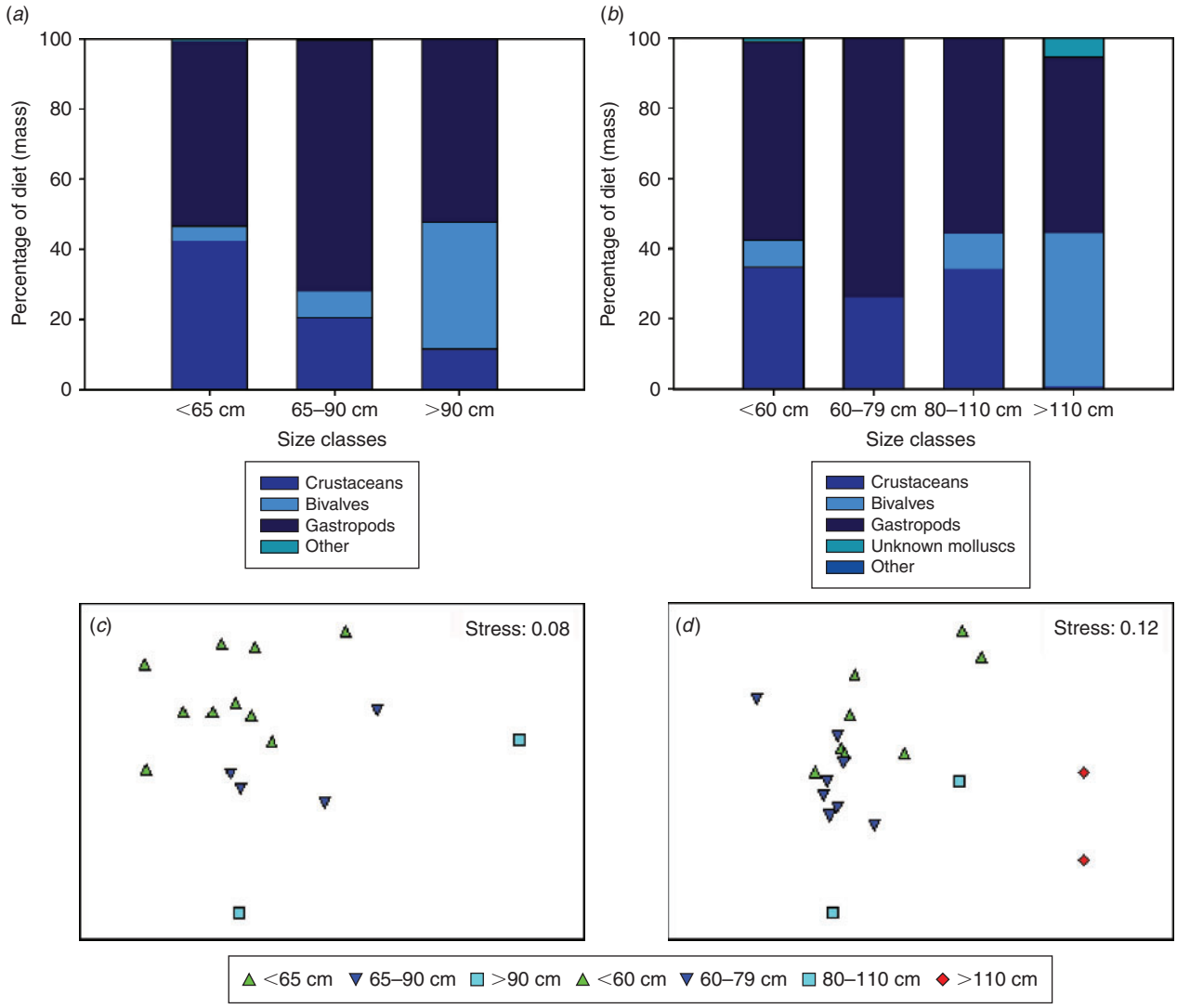

Fig. 3. Mean percentage gravimetric contribution of six prey groups to the diets of (*a*) three Australian size classes ($n = 67$) and (*b*) four size classes overall (Australia and Taiwan, $n = 85$). Non-metric multidimensional scaling ordinations of the mean percentage gravimetric contribution for three Australian size classes ($n = 67$) in (*c*) and four size classes overall ($n = 85$) in (*d*).

the paucity of adult females prevented an accurate determination of female size-at-maturity. From aquarium observations and our data, it seems likely that sexual maturity occurs at a disc width of around 150–160 cm (40–50 kg). More mature individuals will need to be assessed to confirm this estimate.

Male reproduction

Males became sexually mature at disc widths of ~130 cm (25–30 kg). These estimates suggest that maturity is attained at ~50–54% of maximum disc width in females (assuming an asymptotic disc width of 297 cm for both sexes: Schluessel 2008) and 43% in males. In females, these values are comparable to maturity at 56% of total disc width in *Myliobatis californica* (Martin and Cailliet 1988) and 58% in *R. bonasus* (Neer and Thompson 2005) but are lower than in *Manta birostris* (88%) and *Mobula hypostoma* (66%) (Holden 1974). However, maturity of *Manta birostris* may occur at a lower percentage of total disc width, recently estimated to be around 67% (A. Marshall, pers. comm.). Sexual dimorphism in size at sexual maturity is commonly observed in a variety of elasmobranchs (Stevens and McLoughlin 1991; Natanson *et al.* 1995; Capapé *et al.* 2007). This is the first account of sexual dimorphism in *Aetobatus narinari*.

Histological sections of testes, epididymis and ductus deference confirmed the presence of mature sperm in a large male caught in February near Heron Island. If the gestation period of *A. narinari* does indeed last around twelve months in regions with cooler water temperatures (as suggested by aquarium studies, e.g. J. Mahon, F. Chua and P. Newman, unpubl. data), parturition occurs in late summer (as determined by the presence of neonates in Moreton Bay) and mating occurs immediately after parturition (as observed by Uchida *et al.* 1990 at Okinawa Expo Aquarium). Therefore, February would be expected to fall

within the mating season and adult males would be expected to possess mature sperm.

Regional variations in size-at-maturity and gestation

Studies can give conflicting results concerning factors such as gestation period, size-at-maturity, and litter or embryo size for the same species. Insufficient sample size may be one reason for discrepancies, but other factors are likely to contribute. In male *A. narinari* individuals collected from Indonesia, the size at which 50% of the population had reached maturity (DW_{50}) was determined to be 99.8 cm, and the size at which 95% of the population had reached maturity (DW_{95}) was 100.2 cm (White and Dharmadi 2007). In comparison, the present study determined DW_{50} to occur at 130.1 cm and DW_{95} at 131.1 cm. Essentially, the size-at-maturity of Australian and Indonesian males varied by 30 cm.

Data collected from different aquaria showed that water temperature may affect the gestation period in *A. narinari*, with a 10°C increase in ambient temperature effectively halving gestation periods (Table 1). Uchida *et al.* (1990) estimated *A. narinari*'s gestation period to range between 11 and 12 months, and unpublished data from three different aquaria (Table 1) indicate that gestation in *A. narinari* can vary from 180 to 188 days to 331–377 days (J. Mahon, F. Chua and P. Newman, unpubl. data: Uchida *et al.* 1990). In other studies, it was estimated to range between 240 and 365 days (Table 1). Studies in other elasmobranchs have also shown that temperature facilitates developmental rates (Harris 1952; Wallman and Bennett 2006). For example, gestational periods in *Raja eglanteria* varied between two and three months, based on whether specimens were collected from Florida or Delaware (Libby and Gilbert 1960).

If temperature influences embryonic growth and therefore gestation periods, it could also lead to faster growth in juveniles and an earlier onset of maturity. Regional life history variations (e.g. age and size-at-maturity) within the same species have already been found in a range of elasmobranchs, including female *Myliobatus californica* (Herald *et al.* 1960; Martin and Cailliet 1988) and *Rhinoptera bonasus* (Neer and Thompson 2005). Generally, elasmobranch populations inhabiting colder waters seem to mature at (and possibly grow to) larger sizes than their tropical counterparts. Australia and Taiwan represent the southern and northern extents of the geographical range of *A. narinari* and feature much lower annual sea-surface temperatures than Indonesia. Temperature is therefore a likely factor driving different sizes-at-maturity found between these regions.

Litter size

Litter sizes varied between one and 10 embryos in *A. narinari*, with most litters containing only up to four embryos (Table 1). Discrepancies concerning different sizes at birth in *A. narinari* should be interpreted carefully, because *A. narinari* seems to abort its young quite easily (Gudger 1914; V. Schluessel, pers. obs.) and aborted near-term embryos could be mistaken for neonates. On the other hand, if gestation times in tropical regions are shorter and size-at-maturity lower, sizes at birth in these regions should be expected to be lower than in sub-tropical/temperate regions.

Feeding habits

Stomach content analysis showed that feeding in *A. narinari* was quite selective and restricted to the same two taxa within two geographically distinct regions, i.e. Australia and Taiwan. An overwhelming 78.0% (by mass) and 97.9% (by mass) of the dietary intake consisted of molluscs in Australia and Taiwan, respectively, followed by 22.0% (Australia) and 2.1% (Taiwan) of crustaceans. Within these taxa, prey species belonged mainly to gastropods, bivalves and malacostracans (Anomura) and ingestion of other prey, such as polychaetes or echinoderms, was probably accidental. Gastropods characterised the diet of every size class in Australia and Taiwan. Malacostracans were important components of diets of small and medium-sized groups but did not typify diets of large rays. All prey groups contributed to the observed differences in diets, with bivalves generally distinguishing diets of smaller and large animals in Australia, as well as in the combined analysis.

Stomach contents of *Aetobatus flagellum* (Japan) consisted exclusively of a few species of bivalves, and to a much lesser extent, of gastropods (Yamaguchi *et al.* 2005). Diets of related species, e.g. *Myliobatis aquila* in Mediterranean waters (Bini 1967; Capapé 1976; Jardas *et al.* 2004) and *M. californica* in Humboldt Bay, California (Gray *et al.* 1997) also consisted mainly of molluscs (bivalves and gastropods), with smaller contributions of decapods, sipuniculids and polychaetes. In *M. aquila*, 14.5% of all stomachs were found to be empty (Jardas *et al.* 2004), which is similar to the number of empty stomachs encountered in the present study (11.8% in Australia and 12.2% in Taiwan).

Aetobatus narinari seems to be a hard prey specialist that feeds predominantly on shelled animals. As early as 1894, Thurston reported on the damage caused by *A. narinari* to pearl-oyster banks in Ceylon, and both Coles (1913) and Gudger (1910) noted that stomachs of *A. narinari* caught along the south Atlantic coast of the US were entirely filled with clams. Not one shell fragment was ever found in any of the examined stomachs in the present study, or studies by Yamaguchi *et al.* (2005), Gudger (1912, 1914) or Coles (1910). Conversely, the related *R. bonasus*, which also feeds predominantly on molluscs and gastropods (Smith and Merriner 1985), was recently classified as an 'opportunistic generalist' instead of a 'hard prey specialist' (Collins *et al.* 2007) and shells were frequently found in its stomach. Shell fragments were also recorded in stomachs of *R. javanica* and *Myliobatis freminvillei* (Shipley and Hornell 1906; Bearden 1959). The absence of shell fragments combined with the special jaw morphology of *Aetobatus* (Summers 2000) indicate that this genus may be exceptionally suited for consumption of hard-bodied prey and may therefore be classified as a true feeding specialist.

General accounts on the diet of *A. narinari* also report contributions of larger species like teleosts and cephalopods to the overall diet (e.g. Bigelow and Schroeder 1953; Homma *et al.* 1994). As the present study revealed that size-related dietary shifts occur, it is possible that individuals over 200 cm in disc width, which were not included in this study, consume different prey items. In Taiwan, smaller animals are frequently caught in gill-nets and large rays are also caught on teleost-baited long-lines (pers. comm. with fishermen). In the current study,

Aetobatus narinari was found to feed on very common and highly abundant prey with no commercial value. Several oyster farms exist in Moreton Bay, none of which are known to have any protective measures against stingray predation, and no oysters were found in any of the stomachs assessed from Moreton Bay during 2005–2007. Although predation by *Aetobatus* of commercially farmed bivalves has been described by Thurston (1894; predation of oyster beds in Ceylon by *A. narinari*), and Yamaguchi *et al.* (2005; predation of bivalve stocks by *A. flagellum*), it does not seem to be an issue in Moreton Bay. This could indicate that food sources in general are quite abundant within the region.

Acknowledgements

We thank John Page, Stephen Taylor and Sea World (Gold Coast, Australia) for help with animal collections, Justin Hsieh and the Makung Fisheries Institute for assistance in Taiwan and Jenny Giles, Barbara Wueringer, Guy Ely and Kurt Davis for volunteer work in Taiwan and on Heron Island. We also thank Tina Chua and Claire Aland for help with the histology processing, Sebastian Pardo for help with species identification, IRI and Primer analysis and Darryl Potter, Jon Healy and Peter Davie from the Queensland museum for help with the identification of gastropods, bivalves and crustaceans. Lastly, we thank the referees for their valuable comments on the manuscript. V.S. was supported by a DAAD postgraduate scholarship and a University of Queensland postgraduate living stipend. This project was supported by the UQ Graduate School and an Australian Research Council Linkage Grant (LP0214956) to S.P.C. Experiments complied with the guidelines of The University of Queensland Animal Ethics Committee (AEC No: ANAT/781/06/ARC).

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Manuscript received 14 October 2009, accepted 26 May 2010