RESEARCH ARTICLE

Functional implications of ontogenetically and sexually dimorphic dentition in the eastern shovelnose ray, *Aptychotrema rostrata*

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ABSTRACT

Unlike other elasmobranchs, batoids exhibit sexually dimorphic dentition. The functional implications of such dentition, however, remain understudied. For the present study, ontogenetic and sexual dimorphism in tooth and jaw structure, together with the functional implications of this dimorphism, were determined in the eastern shovelnose ray, *Aptychotrema rostrata*. Sexually dimorphic dentition and jaw structure was first observed in sub-adult age classes, with males developing a pronounced lower jaw at the symphysis. Monognathic heterodonty was prominent in mature males, with teeth in the symphyseal region developing significantly greater heights and sharpness ratios in comparison to females. *Ex vivo* mechanical grip strength tests were used to determine simulated bite-grip tenacity. The mean peak pull-out forces required to withdraw a section of a dissected pectoral fin from between jaws closed with a constant occlusal force was highest for mature males, intermediate for mature females and lowest for immature females and males. Although the species exhibits ontogenetic variations in diet, these were unrelated to sex. Rather, the larger and highly cuspidate teeth of mature males increased the bite-grip tenacity, which likely aids in copulation.

KEY WORDS: Bite, Morphology, Jaw, Replacement, Teeth

INTRODUCTION

Many species of elasmobranch develop sexual dimorphism of shared characters through the course of ontogeny. These include both morphological characters, such as total length (Last and Stevens, 2009; Orlov and Cotton, 2011) and head shape (Ellis and Shackley, 1995; Filiz and Taskavak, 2006), and sensory biology characters, such as development of the ampullae of Lorenzini (Crooks and Waring, 2013). Within the batoid fishes (skates, rays and guitarfishes), a known sexual dimorphism occurs in dentition, as males of many species, in contrast to females, develop highly cuspidate teeth as they mature (Feduccia and Slaughter, 1974; Kajiura and Tricas, 1996; Powter et al., 2010; Taniuchi and Shimizu, 1993).

Initially, it was thought that sexually dimorphic dentition was due to the sexes occupying different niches and targeting different prey (Feduccia and Slaughter, 1974). However, support for this hypothesis is lacking, as data from four species of rajids that exhibited sexually dimorphic dentition showed no significant difference between the diets of mature males and females (Lyle, 1983; McEachran, 1977; Taniuchi and Shimizu, 1993). Rather, the change in tooth morphology is thought to enhance a male's ability to grip a female's fin during mating, in order to temporarily hold

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their bodies in a position to facilitate clasper insertion (Kajiura et al., 2000; Stevens, 1974; Tricas and Lefeuvre, 1985; Young, 1993). This hypothesis was supported by analysing the dentition of the Atlantic stingray, *Dasyatis sabina*, a species that exhibits sexually dimorphic dentition. Biomechanical grip strength tests demonstrated that the teeth of mature male *D. sabina* had significantly higher grip tenacity in comparison with mature females (Kajiura and Tricas, 1996).

In batoids that display sexually dimorphic dentition, mature males may demonstrate pronounced monognathic heterodonty, in which the anterior teeth develop elongated cusps while the more lateral teeth remain more molariform (Kajiura and Tricas, 1996; Taniuchi and Shimizu, 1993). In contrast, mature females retain their more molariform dentition throughout the life cycle (McEachran, 1977; Taniuchi and Shimizu, 1993). Given that many species of ray consume a variety of benthic prey, including hard-bodied invertebrates, molariform dentition enables the individual to crush and grind their prey (Collins et al., 2007; Sasko et al., 2006). Therefore, the development of dentition with increased anterior cuspidation in mature males is likely to enhance their ability to grip a female, yet still allow the ingestion of a wide variety of prey. Indeed, for *D. sabina*, mature males only developed sexually dimorphic dentition during the breeding season, reverting to molariform dentition outside this time (Kajiura and Tricas, 1996).

Batoids, in common with all other elasmobranchs, have numerous tooth rows that are attached to a fibrous connective tissue sheet, rather than being directly fused to the jaw (Dean et al., 2008; Frazzetta, 1994; Motta and Wilga, 2001). This arrangement allows for continuous tooth replacement in which tooth buds produce new teeth on the lingual side of the jaw that develop as they move labially, with the oldest and often worn or damaged teeth falling out at the front of the mouth (Compagno et al., 2005; James, 1953; Moss, 1972). Serial tooth replacement also allows for the production of larger teeth as an animal grows (Moss, 1972), as well as changes in tooth morphology through ontogeny or even seasonally (Kajiura and Tricas, 1996). The rate of tooth replacement varies among species, with previous studies demonstrating that teeth can move one series in the labial direction every 8 to 28 days (Boyne, 1970; Frazzetta, 1994; Luer et al., 1990; Moss, 1967; Reif et al., 1978).

Although sexually dimorphic dentition has been observed in a number of batoid families, data are lacking for the Rhinobatidae (shark-like rays). This group is of interest because they have evolved a body form that differs from other flat-bodied batoids, yet they still occupy similar ecological niches. Additionally, rhinobatids have a relatively rigid body in comparison with other batoids, which may add to the difficulties of mature males securing a receptive mate. The present study aimed to examine ontogenetic changes in tooth and jaw morphology, as well as the biomechanical significance of sexual dimorphic dentition (Kajiura and Tricas, 1996), in the eastern shovelnose ray, *Aptychotrema rostrata* (Shaw and Nodder 1794). To achieve these aims, we determined the tooth replacement rate and the changes in tooth and jaw morphology from individual rays

List of symbols and abbreviations

JH_{lower}	height of the lower jaw at the symphysis
JH_{upper}	height of the upper jaw at the symphysis
JW	jaw width
M_b	body mass
$P_{0\%}$	symphysis
$P_{15\%}$	15% of the distance from the symphysis to the lateral border of the tooth band
$P_{75\%}$	75% of the distance from the symphysis to the lateral border of the tooth band
$P_{100\%}$	100% of the distance from the symphysis to the lateral border of the tooth band
SEM	scanning electron microscopy
TBW	tooth band width
T_{erupt}	lingual 'tooth eruption' point
TH	tooth height
TL	total length
T_{loss}	labial 'tooth loss' point
$T_{occlusal}$	occlusal surface
TR	tooth row count
TSR	tooth sharpness ratio
TW	tooth width

representing a range of maturity classes, and conducted biomechanical tests to examine whether dental morphology influenced simulated bite grip strength. Overall, we tested the hypothesis that the sexually dimorphic dentition of mature males increased their grip tenacity in comparison to juvenile male rays and female rays.

RESULTS

Length versus mass relationship
The body mass (M_b) of *A. rostrata* increased close to the cube of total length (TL; mm) (Table 1, Fig. 1A). The equations describing the relationships between TL and M_b were: $TL=65.0M_b^{0.344\pm0.003}$ (R^2=0.992, N=133, P<0.05) for males and $TL=66.5M_b^{0.338\pm0.0025}$ (R^2=0.993, N=138, P<0.05) for females.

Jaw morphometry
Best-fit least-squares regression analysis showed that jaw width (JW) was positively correlated with TL for both females and males (Fig. 1B). Additionally, ANCOVA indicted that there were no significant differences between the slopes and magnitudes of the regression lines between the sexes (d.f.=1, P>0.05). As with JW, there was no significant difference between the upper jaw height (JH_{upper}) of females and males (ANCOVA, d.f.=1, P>0.05), with JH_{upper} increasing in a linear fashion for both sexes (Fig. 1D). Unlike JW and JH_{upper}, sexual dimorphism was seen in lower jaw height (JH_{lower}), as the JH_{lower} of mature males had an increased girth at the symphysis. While JH_{lower} retained a linear relationship with TL for females, male JH_{lower} data were best described by an exponential equation (Table 1), with the regressions between females and males being significantly different (ANCOVA, d.f.=1, P<0.05). The JH_{lower} of males began to increase disproportionately at the onset of sexual maturity at approximately 600 mm TL (Fig. 1C), with the medial third of the lower jaw showing the most pronounced increase.

Tooth morphometry
Aptychotrema rostrata exhibited gradient monognathic heterodonty, in which tooth morphology gradually changed from the symphyseal region to the laterals. This condition was highly pronounced in mature males. For both sexes, the crown of symphyseal teeth had a relatively elongated, inward-directed cusp that was large in anterior teeth, reduced on the intermediates and 'keel-like' on the laterals

Table 1. Equations describing the relationships between body mass (M_b), jaw width (JW) and jaw height (JH) in relation to total length (TL) for male and female *Aptychotrema rostrata*

Equation	Sex	R^2	N	P
$TL=65.0M_b^{0.344\pm0.003}$	Male	0.992	133	<0.05
$TL=66.5M_b^{0.338\pm0.0025}$	Female	0.993	138	<0.05
$JW=0.302+(0.109TL)$	Male	0.972	139	<0.05
$JW=0.448+(0.108TL)$	Female	0.975	140	<0.05
$JH_{upper}=-0.156+(0.0068TL)$	Male	0.865	140	<0.05
$JH_{upper}=-0.075+(0.0067TL)$	Female	0.881	140	<0.05
$JH_{lower}=1.693+[2.045\times10^{-5}(TL)^2]$	Male	0.920	140	<0.05
$JH_{lower}=-0.691+(0.0146TL)$	Female	0.945	140	<0.05

(the commissural teeth) (Fig. 2). The laterals were 'pavement-like' in appearance compared with those closer to the symphysis (Fig. 2). Additionally, teeth in the lower jaw close to the symphysis in mature males appeared to lose the sharp edge on the lateral surface that was observed in the equivalent teeth of mature females (Fig. 2D).

Increases in JW were accompanied by increases in tooth band width (TBW) on both the upper and lower jaws. Such increases were a function of a significant increase in the number of tooth rows between immature and mature individuals (one-way ANOVA, d.f.=3, P<0.05; Table 2).

The teeth at position $P_{15\%}$ (15% of the distance from the symphysis to the lateral border of the tooth band) on either side of the symphysis in 140 female and 140 male rays showed a linear relationship between tooth width (TW) and increasing TL (Fig. 3A). ANCOVA demonstrated that there was no significant difference between TW and TL for females and males (d.f.=1, P>0.05). For tooth height (TH) and tooth sharpness ratio (TSR), however, there was a significant difference in growth between females and males (ANCOVA, d.f.=1, P<0.05; Fig. 3B,C). The differences between the sexes were due to an increase in TH and TSR in males around the onset of sexual maturity (Fig. 3B,C). TSRs at $P_{15\%}$ increased from approximately 0.6 in the smallest rays of both sexes to approximately 0.8 in mature females and 1.4 in mature males. A visual change in TSR is apparent in Fig. 4.

Analysis of TW, TH and TSR from $P_{15\%}$ for the maturity classes immature, sub-adult and mature showed significant differences among groups (ANOVA, P<0.05; Fig. 5). Mature males were significantly different to all size classes of females and to immature males for all parameters (Tukey *post hoc*, P<0.05). For TH and TSR, mature males and sub-adult males were not significantly different to one another (Tukey *post hoc*, P>0.05), but were significantly different to all other groups (Tukey *post hoc*, P<0.05; Fig. 5). Overall, these results demonstrated that sexually dimorphic dentition became apparent in the sub-adult size classes of *A. rostrata*.

Effect of tooth position on tooth sharpness ratio
Two-way ANOVA demonstrated that TSR at $P_{0\%}$, $P_{15\%}$, $P_{75\%}$ and $P_{100\%}$ were significantly different at the lingual 'tooth eruption' point (T_{erupt}), the occlusal surface ($T_{occlusal}$) and the labial 'tooth loss' point (T_{loss}) (d.f.=3, P<0.05; Fig. 6). Within-subjects factorial *post hoc* tests demonstrated that the TSR of mature males was significantly different from all other maturity stages at all locations and positions along the jaw (d.f.=3, P<0.05), except for position $P_{100\%}$ (d.f.=3, P>0.05). This is evident from scanning electron microscopy (SEM) photographs, as mature males developed a highly cuspidate and conical tooth form in comparison with other size classes (Fig. 4). The highest mean values of TSR for mature males occurred from $P_{15\%}$ at all locations (Fig. 6), evident in the SEM photographs (Fig. 2). Overall, these results indicate the presence of sexual
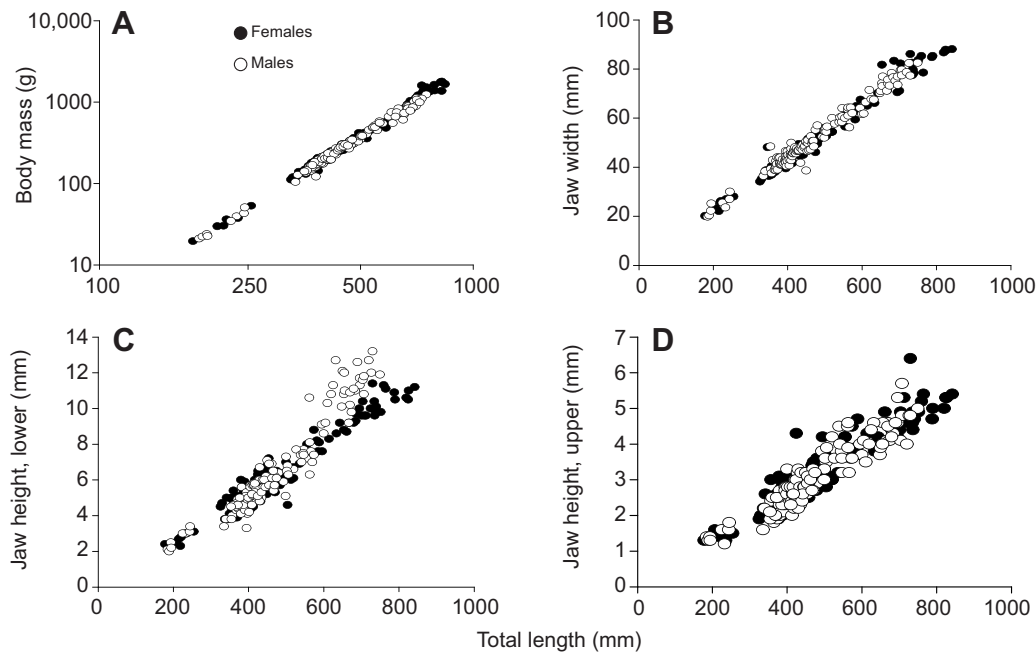
Fig. 1. Scatter plots demonstrating the relationships between total length (mm) and morphological characters in *Aptychotrema rostrata*. Data shown are (A) body mass (g), (B) jaw width (mm), (C) lower jaw height (mm) and (D) upper jaw height (mm) for female (closed circles) and male (open circles) *A. rostrata*.

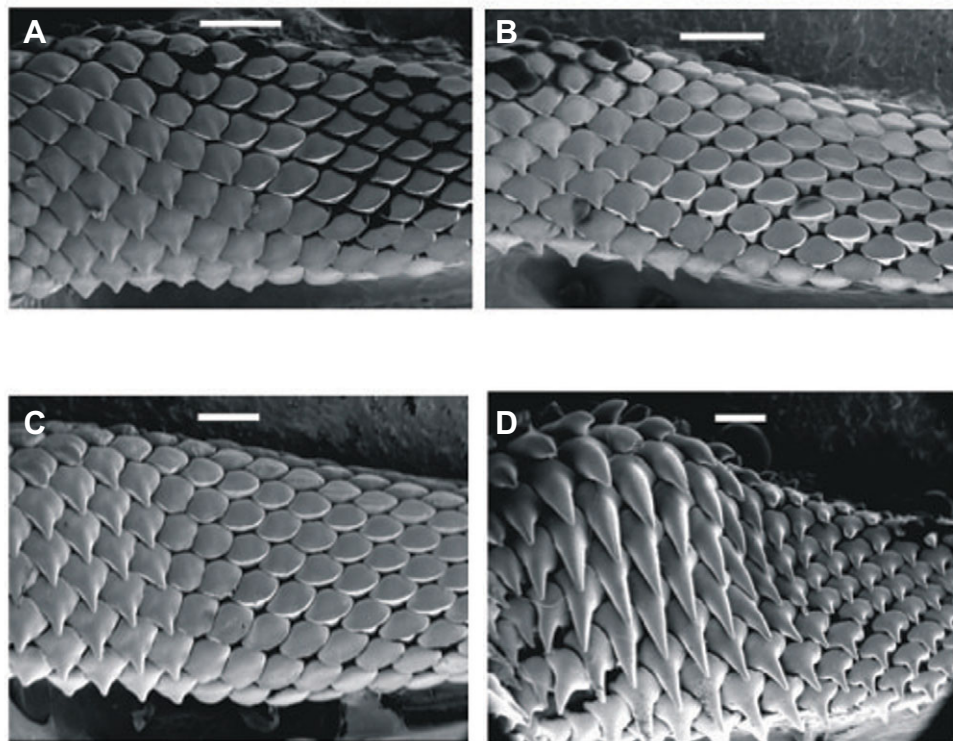dimorphic dentition in all locations of the lower jaw, with the exception of teeth at $P_{100\%}$.

Tooth replacement rate

Mean (±s.d.) tooth replacement rate for rays maintained in seawater at 21–24°C was 13.61±1.25 days series^{-1} for mature rays (*N*=9) and 10.40±0.89 days series^{-1} for immature rays (*N*=6).

Bite-grip tenacity tests

Measurable forces additional to the intrinsic friction of the testing apparatus (≤0.05 N) were generated when occluded jaws resisted

the labial translation of the pectoral fin (Fig. 7). Force–displacement plots indicated a build-up of force as fin withdrawal was initiated. Minor slippage, indicated by sudden falls in force, occurred in all groups, although it was most pronounced in the immature categories, where force never built up to the levels seen in larger individuals, particularly for males (Fig. 7). Mature females were characterised by minor slippages and re-engagements, with a gradual build-up in force followed by a loss of grip. The teeth of mature males demonstrated firm re-engagement of the fin following any slippage, allowing for larger forces to be realised (Fig. 7).

Fig. 2. Scanning electron micrographs showing the ontogenetically and sexually dimorphic dentition in the lower jaw of *Aptychotrema rostrata* to the lateral right of the symphysis. (A) Immature female, total length (TL)=582 mm; (B) immature male, TL=570 mm; (C) mature female, TL=820 mm; (D) mature male, TL=750 mm. This region of the jaw highlights the sexually dimorphic condition. Monognathic heterodonty is clearly evident laterally from the symphysis. Scale bars, 1 mm. The jaw symphysis is on the left of all images.

Table 2. Mean jaw measurements taken from a sub-sample of mature female (N=8), mature male (N=8), immature female (N=4) and immature male (N=4) *Aptychotrema rostrata*

Measurement	Mature females	Mature males	Immature females	Immature males
TBW_{lower}	41.45±4.23[a]	40.88±3.12[a]	30.20±5.34[b]	30.73±4.80[b]
TBW_{upper}	43.02±3.95[a]	41.08±5.24[a]	31.45±6.04[b]	31.73±4.96[b]
TR_{lower}	67.63±2.88[a,b]	74.13±6.08[a]	60.25±8.22[b]	59.00±4.3[b]
TR_{upper}	73.13±2.64[b]	82.13±4.39[a]	64.75±8.98[b,c]	64.50±5.81[c]

Measurements are the tooth band width on the lower (TBW_{lower}) and upper jaws (TBW_{upper}) and the number of tooth rows on the lower (TR_{lower}) and upper jaw (TR_{upper}). Different superscripted letters indicate significant differences between maturity stages for a given measurement.

The frictional forces resisting withdrawal of the fin from between the jaws decreased in the following order: mature males>>mature females>immature females=immature males (Table 3). The mean peak pull-out forces for mature males were significantly greater than all other categories (ANOVA, $P<0.05$).

DISCUSSION

The present study provided the first detailed investigation for sexually dimorphic dentition in a rhinobatid. *Aptychotrema rostrata*

Fig. 3. Scatter plot demonstrating the relationship between total length (TL) and various tooth parameters for female (closed circles) and male (open circles) *Aptychotrema rostrata*. (A) Tooth width; (B) tooth height; (C) tooth sharpness ratio. Tooth measurements were taken from positions 15% of the distance from the symphysis to the lateral border of the tooth band ($P_{15\%}$).

developed sexually dimorphic dentition with ontogeny, as the dentition of mature males was highly cuspidate in comparison with mature females. This increased cuspidation became present in sub-adult males and was pronounced in regions of the lower jaw in close proximity to the symphysis. A functional implication of the observed sexually dimorphic condition was an increased grip tenacity of mature males, supporting findings from a different batoid species (Kajiura and Tricas, 1996).

Although TW increased with ontogeny, the increases could not account for the increased TBW observed in the lower and upper jaw. Rather, the difference in TBW was due to an increase in the number of tooth rows, ranging from approximately 25 in small juveniles to approximately 40 in large adults. Although such differences in tooth count seem to be a common feature of many batoids and sharks with numerous small teeth (Applegate, 1965; Ellis and Shackley, 1995), the relative paucity of ontogenetic studies of elasmobranch dentition precludes any widespread trend to be determined. As tooth count can be used as a taxonomic tool to assist in the identification of elasmobranchs (e.g. Compagno et al., 2005; Last and Stevens, 2009; White et al., 2005), possible ontogenetic changes therefore need to be considered.

Monognathic heterodonty, where more than one tooth morphology exists within a jaw, is a fairly common feature in demersal elasmobranchs (Herman et al., 1997) and is particularly distinctive in heterodontiform sharks, such as *Heterodontus portusjacksoni* (Compagno et al., 2005; Nobiling, 1977; Powter et al., 2010). Heterodonty presumably has functional implications, with pointed teeth better suited for piercing and gripping while the more flattened teeth are better for crushing and grinding (Whitenack and Motta, 2010, Ramsay and Wilga, 2007). In *A. rostrata*, the laterals are the more flattened teeth, which corresponds to the location where compressive forces would be highest during jaw closure (Summers, 2000). However, as noted in other elasmobranch species, the increased cuspidation in symphyseal teeth may allow for the restraint of soft-bodied prey items (Dean et al., 2008; Ramsay and Wilga, 2007) or for gripping of a mate during copulation (Kajiura and Tricas, 1996; McEachran, 1977).

Aptychotrema rostrata exhibited monognathic and ontogenetic heterodonty, with regional differences in tooth morphology increasing through ontogeny, with the tooth cusp of more medial teeth becoming increasingly elongated. Other members of the family Rhinobatidae have also been shown to exhibit ontogenetic heterodonty (e.g. *Rhinobatos rhinobatos*, *Zanobatos schoenleinii*, *Platyrhina sinensis*); however, it is absent in members of the Rhinidae (*Rhina ancylostoma*) and Rhynchobatidae (e.g. *Rhynchobatus djiddensis*) (Herman et al., 1997).

In addition to these ontogenetic changes, JH_{lower}, TH and TSR demonstrated diverging morphologies between the sexes. This first became apparent in *A. rostrata* at a body size of approximately 600 mm TL, corresponding to the onset of male sexual maturation (Kyne and Bennett, 2002b). Similar patterns of dimorphism are reported in *Dasyatis akajei* (Taniuchi and Shimizu, 1993) and
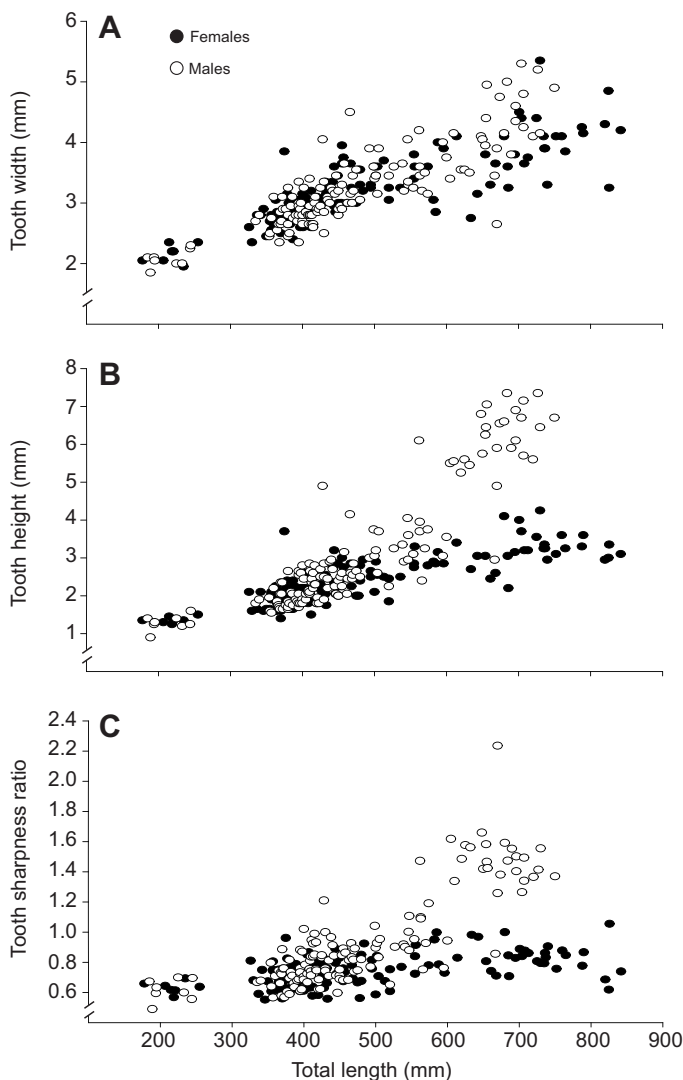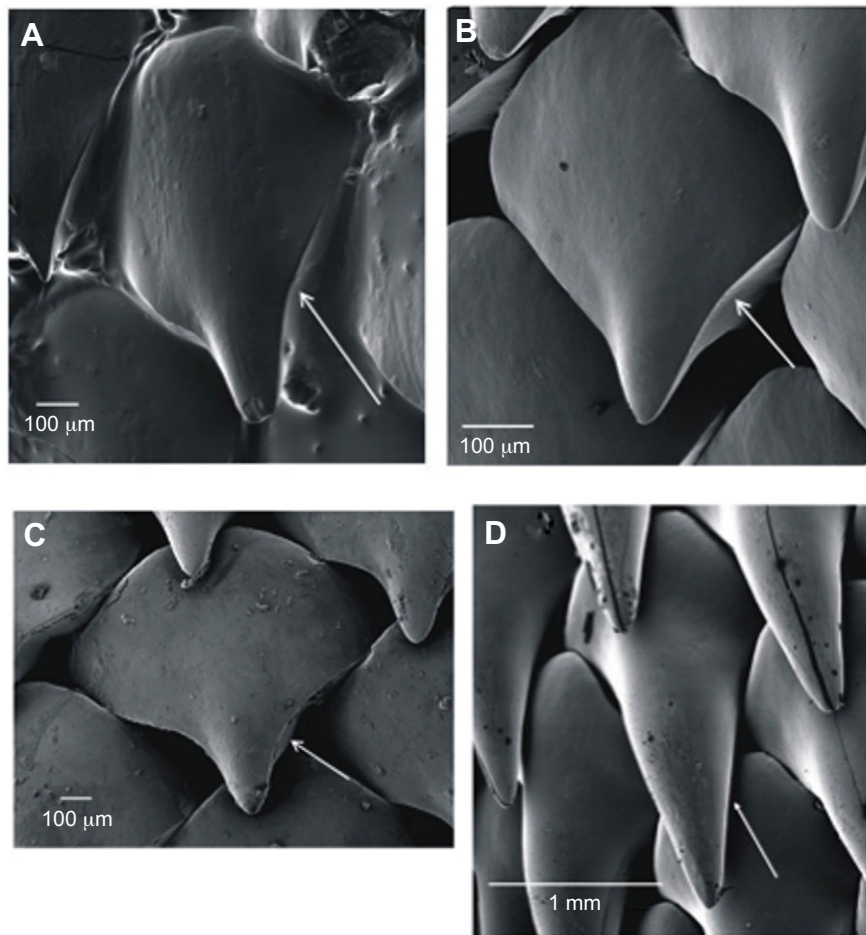
Fig. 4. Scanning electron micrographs of teeth from different maturity stages of *Aptychotrema rostrata*. Images show a typical tooth from $P_{15\%}$, right of the lower symphysis, from (A) an immature female (TL=634 mm), (B) an immature male (TL=520 mm), (C) a mature female (TL=680 mm) and (D) a mature male (TL=727 mm). The mature male appears to lose the cutting surface (indicated by arrow) on symphyseal teeth that was typical of other maturity classes.

Scyliorhinus canicula (Ellis and Shackley, 1995), where male dentition deviates from a common growth trajectory around the time of sexual maturation.

While ontogenetic shifts in diet have been identified in *A. rostrata* (Kyne and Bennett, 2002a), there is no evidence of sexually divergent dietary intake among adult rays (M.B.B., unpublished). These outcomes suggest that while jaw size may be important in terms of the maximum size of prey items that can be taken, the sexually dimorphic dentition would play a relatively minor role in feeding for the species. Therefore, it is likely that the sexually dimorphic dentition of *A. rostrata* allows mature males to grip a female for reproductive behaviour, yet still enables them to capture a wide variety of prey items, including hard-bodied invertebrates. However, further investigation is required to test whether sexually dimorphic dentition influences feeding performance.

The onset of sexually dimorphic dentition parallels the development of other secondary sexual characteristics in batoids, notably an increase in clasper length (Kyne and Bennett, 2002b; Taniuchi and Shimizu, 1993). This synchrony of development implies that the dentition may have a role in reproductive behaviour. Although the mating behaviour of *A. rostrata* has not been documented, it is likely to involve a pre- (and probably mid-) copulatory biting phase similar to that reported in many other elasmobranch species (Carrier et al., 1994; Johnson and Nelson, 1978; Kajiura et al., 2000; Klimley, 1980; McCourt and Kerstitch, 1980; Nordell, 1994; Tricas, 1980; Tricas and Lefeuvre, 1985; Young, 1993). Evidence for mating behaviour was witnessed during the study, as mature females both in the laboratory and during field collections had visible mating scars on the pectoral fins and cranial

areas (A.N.G., personal observation). Clasper insertion is necessary for internal fertilisation in all chondrichthyan fishes and is commonly facilitated by the male using his jaws to grip parts of a receptive female's body, often a pectoral fin. It has been suggested that for species that exhibit predominantly molariform dentition, males have evolved a sexually dimorphic dentition in order to restrain a female during copulation (McEachran, 1977; Taniuchi and Shimizu, 1993).
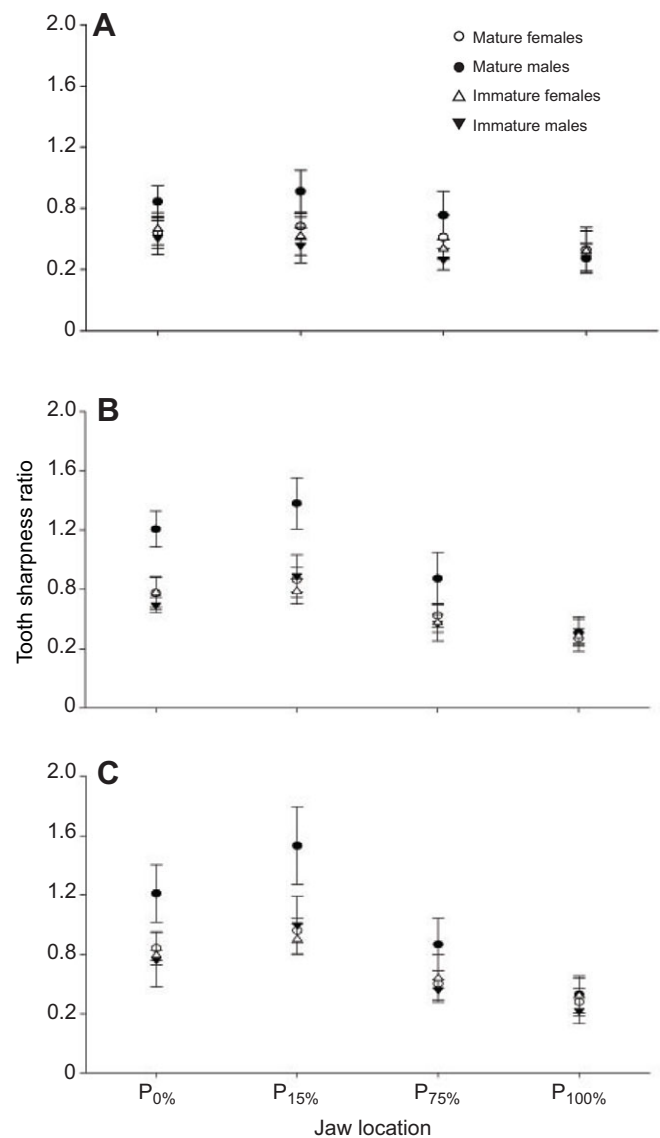
In male *A. rostrata*, the increased girth of the lower jaw at the symphysis could act to localise occlusal (bite) forces in this region. In turn, this localisation could facilitate the required grip of the pectoral fin tissue by the long, pointed teeth (Whitenack and Motta, 2010). During copulatory behaviour, the highly cuspidate teeth may become 'interlocked' within the dermal denticles of the female, functionally analogous to the interaction between arthropod claw tips and engaged surfaces (Han et al., 2011). The *ex vivo* grip tenacity results in the present study showed that the mean peak forces required to draw pectoral fin specimens out of the mouth (each closed with the same occlusal force) was correlated with tooth and possibly jaw morphology. The sharp cuspidate teeth characteristic of mature males, particularly on the occlusal surface, provided a significantly stronger grip than immature males and all females. These results are similar to those reported for *D. sabina*, where the sexual dimorphic dentition of mature males had grip tenacities nearly double those of mature females (Kajiura and Tricas, 1996).

In *D. sabina*, variations in male tooth morphology were observed to occur on a seasonal basis. The timing of this was consistent with the hypothesis that sharper teeth develop to facilitate mating and that the dimorphism might be influenced by reproductive steroid
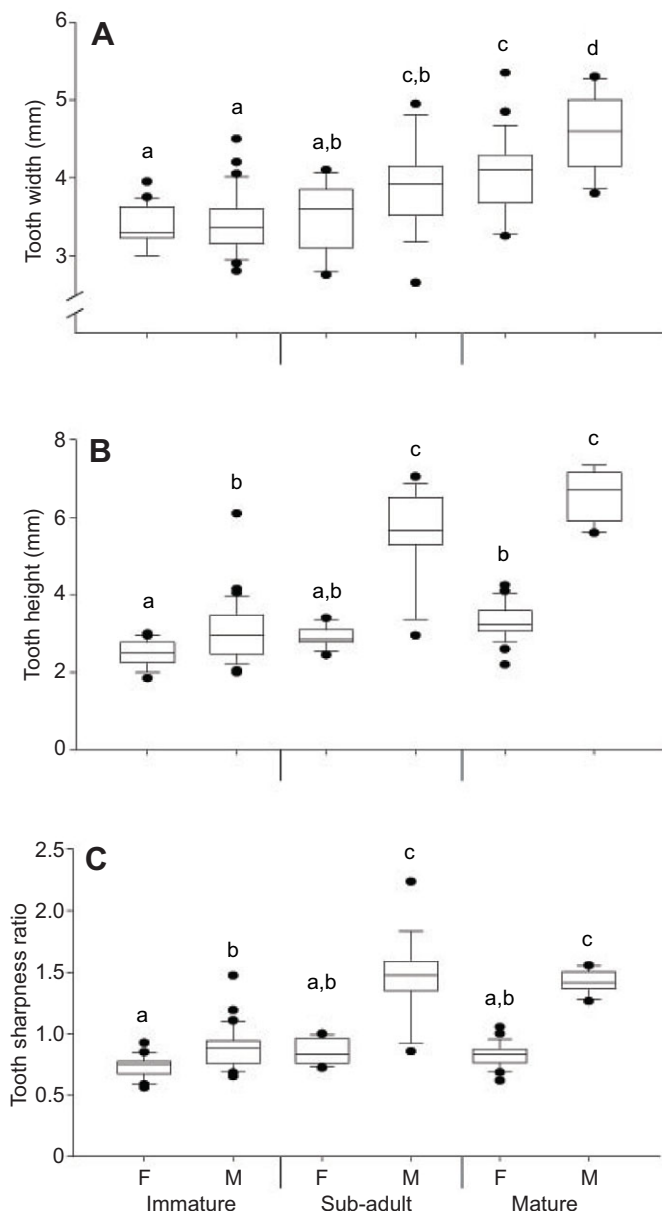
Fig. 5. Box plots demonstrating the median values of (A) tooth width, (B) tooth height and (C) tooth sharpness ratio for immature, sub-adult and mature size classes of *Aptychotrema rostrata*. Boxes represent 25th and 75th percentiles, with the horizontal lines representing the median value. Whiskers above and below boxes represent the 5th and 95th percentiles, respectively. Circles above and below whiskers represent outliers. Letters above each box represent the significance category.

Fig. 6. Scatter plot demonstrating the mean tooth sharpness ratios for mature female (open circles), mature male (closed circles), immature female (open triangles) and immature male (closed triangle) *Aptychotrema rostrata*. The positions of measured teeth at $P_{0\%}$, $P_{15\%}$, $P_{75\%}$ and $P_{100\%}$ (see Fig. 8) are shown in terms of the location of (A) the eruption point (T_{erupt}), (B) the occlusal point ($T_{occlusal}$) and (C) the loss point (T_{loss}) (see Fig. 9).

hormone cycles (Kajiura and Tricas, 1996). Similar seasonal changes in male tooth morphology might have been expected in *A. rostrata*, with its single, clearly defined and relatively short mating season (Kyne and Bennett, 2002b). However, the timing of dimorphism that arises at sexual maturity could not be assessed in the present study, as specimens were collected across a narrow temporal scale. If seasonal variations in tooth morphology were present in *A. rostrata*, as with *D. sabina* (Kajiura and Tricas, 1996), the dental changes would occur well before the onset of mating activity for dentition to become functional (i.e. occlusal). In *A. rostrata* it would take approximately 3 months for this to occur, given the rate of tooth series replacement. However, given that the

onset of sexually dimorphic dentition arose in sub-adult male *A. rostrata*, the dimorphic condition likely remains for the remainder of their life cycle. At present, it remains to be tested whether there is a causal relationship between circulating levels of gonadal steroids and tooth morphology in any species of elasmobranch. Such data would be useful in determining the underlying drivers for sexually dimorphic dentition in batoids, with this type of research encouraged for future investigations.

The present study provides the first detailed observations of sexually dimorphic dentition and the functional implications of this dimorphism in a rhinobatid. The results support previous observations made for other species of batoids and, indeed, the sexually dimorphic condition reflects that of some species of benthic shark (Ellis and Shackley, 1995; Powter et al., 2010). Given that batoids and benthic sharks occupy similar niches, it is therefore
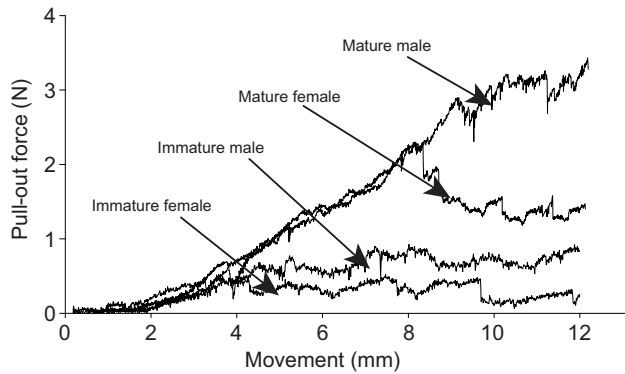
Fig. 7. Graphical representation of the pull forces (N) generated from a mature male, mature female, immature male and immature female *Aptychotrema rostrata* during *ex vivo* biomechanical tests. The sudden drop of mature females trace was due to the loss of grip on the dissected pectoral fin.

likely that the dentition of mature males in such species has evolved to overcome the challenges of both securing a receptive mate and consuming a wide variety of benthic prey. As such, future investigations into the extent of dental sexual dimorphism are encouraged, with a particular emphasis on comparing a variety of benthic species that occupy comparable habitats.

MATERIALS AND METHODS
Specimen collection
Jaws were taken from 280 *A. rostrata* specimens, collected as bycatch in a southeast Queensland trawl fishery. Prior to dissection, the date of capture, body mass (M_b), total length (TL) and state of sexual maturity were recorded. Soft tissues surrounding the jaws were removed, followed by immersion in 3% hydrogen peroxide solution for 30–60 min, and then rinsed with tap water, air-dried and stored in a dry atmosphere.

This study was conducted in accordance with the University of Queensland Animal Ethics Committee guidelines and under General Fisheries Permit PRM03951I.

Morphological measurements
Measurements of various jaw dimensions were made using digital vernier callipers (±0.1 mm; Mitutoyo, Takatsu-ku, Kawasaki, Japan) on 140 females (TL=178–842 mm; M_b=19.5–1768 g) and 140 males (TL=185–750 mm; M_b=20.3–1233 g; Table 4). Sexual maturity status was determined following Kyne and Bennett (Kyne and Bennett, 2002b). The following measurements were taken: overall jaw width (JW), upper jaw height (JH_{upper}) at the symphysis ($P_{0\%}$) (Fig. 8A) and lower jaw height (JH_{lower}) (Fig. 8B). JH_{upper} and JH_{lower} were taken in an anteroposterior direction, from the most anterior functional tooth series to the posterior margin of the jaw at $P_{0\%}$. For the purposes of this study, a tooth series was defined as a line of teeth that runs along and approximately parallel to the jaw cartilage (Compagno, 1988). For each specimen, tooth width (TW) and height (TH) of two teeth were measured using a stereo dissection microscope (Wild M3Z Type-S, Herrbrugge, Switzerland) using a calibrated eyepiece graticule. The teeth measured, one from either side

Table 3. Mean peak pull-out forces determined for bite-grip strength tests in *Aptychotrema rostrata*

Sex	Age class	N	Mean peak pullout force (N; ±s.d.)	Significance
Female	Immature	3	0.63±0.14	a
	Mature	4	1.48±0.59	b
Male	Immature	3	0.62±0.05	a
	Mature	6	2.82±0.36	c

Categories that share a common letter are not significantly different (*P*>0.05) to each other.

Table 4. Group designations of maturity status for male and female *Aptychotrema rostrata* based on total length (TL)

| Maturity status	Male TL (mm)	N	Female TL (mm)	N
Neonate	<450	76	<450	81
Immature	450–599	37	450–539	22
Sub-adult	600–680	16	540–660	13
Mature	>680	11	>660	24

Data are from Kyne and Bennett (Kyne and Bennett, 2002b). Neonates were considered as immature for data analysis.

of $P_{0\%}$, were in a position *ca.* 15% ($P_{15\%}$) of the distance from the symphysis to the lateral border of the tooth band (where the whole tooth band was defined as the linear distance between the most lateral teeth on either side of the jaw) and two tooth series lingual to the occlusal series (Fig. 8B). The mean values of TH and TW were determined from each specimen, with mean TH subsequently divided by mean TW to produce a dimensionless expression – the tooth sharpness ratio (TSR) – for both females and males. The position $P_{15\%}$ was chosen based on previous studies indicating that sexually dimorphic dentition was most pronounced in the symphysal region (Ellis and Shackley, 1995; Kajiura and Tricas, 1996; Taniuchi and Shimizu, 1993).

Measurement of the tooth band width on lower and upper jaws (TBW_{lower} and TBW_{upper}) was taken from a haphazard sub-sample of mature females (*N*=8), mature males (*N*=8), immature females (*N*=4) and immature males
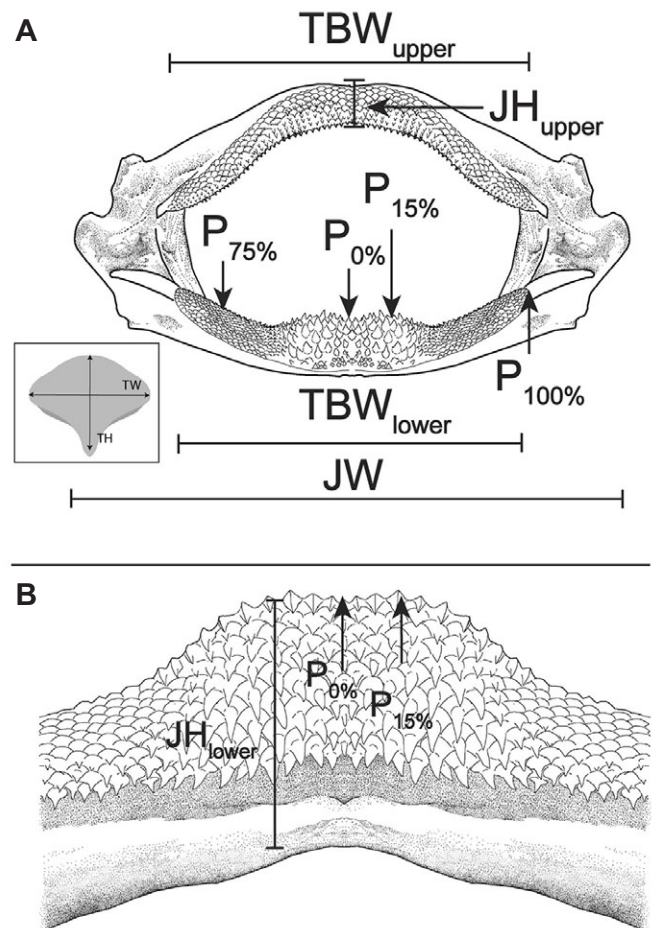
Fig. 8. Diagram indicating the measurements taken from *ex vivo* jaws of *Aptychotrema rostrata*. (A) Overall jaw width (JW), tooth band width on lower and upper jaws (TBW_{lower} and TBW_{upper}), and upper jaw height (JH_{upper}) at the symphysis ($P_{0\%}$). Inset represents the location on each tooth where tooth height (TH) and tooth width (TW) were measured. (B) Lower jaw height (JH_{lower}) at $P_{0\%}$. Teeth measured for tooth sharpness ratio were taken at $P_{0\%}$ and at *ca.* 15% ($P_{15\%}$), 75% ($P_{75\%}$) and 100% ($P_{100\%}$) of the distance from the symphysis to the lateral border of the tooth band on the lower jaw, represented on A.
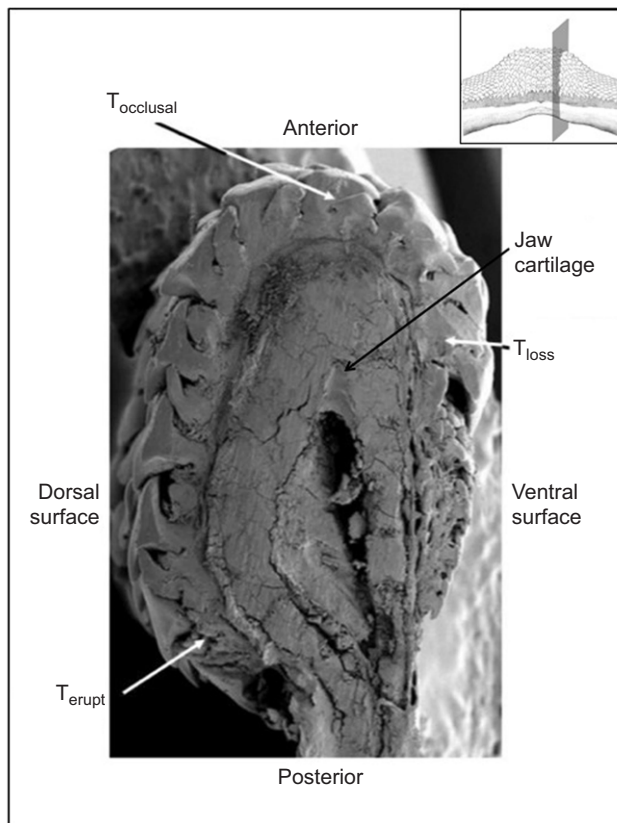
Fig. 9. Scanning electron micrograph demonstrating where tooth sharpness ratios were determined for teeth at three locations in a sagittal plane: the lingual 'tooth eruption' point (T_{erupt}), the occlusal surface ($T_{occlusal}$) and the labial tooth 'loss point' (T_{loss}). The inset represents the area on the jaw from where the cross-section was taken.

(N=4). From this sub-sample, the tooth row counts on the lower and upper jaws (TR_{lower} and TR_{upper}) were also determined for each specimen. For this study, a tooth row was defined as a single replicating line of teeth transverse to the longitudinal axis of the jaw cartilage (Compagno, 1988). Lastly, as well as from $P_{15\%}$, described above, TSR was also determined from this subset of animals at positions $P_{0\%}$ and 75% ($P_{75\%}$) and 100% ($P_{100\%}$) of the distance from the symphysis to the lateral border of the tooth band (Fig. 8A). Measurements of TSR at $P_{0\%}$, $P_{15\%}$, $P_{75\%}$ and $P_{100\%}$ occurred at three locations along each tooth row: the lingual 'tooth eruption' point (T_{erupt}), the occlusal surface ($T_{occlusal}$) and the labial 'tooth loss' point (T_{loss}) (Fig. 9).

Scanning electron microscopy

To gain a visual representation of the dentition of *A. rostrata*, single jaw specimens from each sex and maturity state were examined with SEM. Following dissection, a diamond wafering-saw (LECO VC-50, St Joseph, MI, USA) was used to section jaw specimens in preparation for SEM. Specimens were mounted on aluminium stubs using carbon tape, sputter-coated with platinum (Eiko IB-5 Platinum Sputter Coater, Tokyo, Japan) and viewed with a scanning electron microscope (JEOL JSM 6400F, Tokyo, Japan). Digital micrographs were processed using CorelDraw (v.7.0, Ottawa, ON, Canada).

Tooth replacement rate

Tooth replacement rate was determined in 15 individuals (nine mature males, five immature males and one immature female). Rays were collected from Moreton Bay, Queensland, Australia, using rod and line techniques. They were transported in aerated seawater and held in aquaria at the University of Queensland throughout the time of the study. Tooth replacement rate was determined with individuals anaesthetised using 0.15 g l^{-1} tricaine methane sulphonate (MS-222; Sigma-Aldrich, Castle Hill, NSW, Australia) dissolved in seawater. Rays were then oriented with their ventral surface facing upwards

under a dissection microscope, with a constant flow of aerated seawater (300 ml min^{-1}) containing 0.05 g l^{-1} MS-222 passed over the gills to maintain anaesthesia. A hand-held drill fitted with a flexible shaft and a 0.8 mm engraving cutter (MultiPro 395JC; Flex-Shaft 225; cutter 105, Dremel, Berlin, Germany) was used to grind down individual teeth of the lower jaw in the series prior to the occlusal series. Digital images were taken using a digital camera (Coolpix 995, Nikon, Tokyo, Japan) mounted on a dissection microscope (Wild M3Z Type-S). Animals were allowed to recover, and were returned to a recirculating seawater aquarium on completion of tooth grinding. Tooth replacement rate (teeth day^{-1}) was calculated by reference to a sequence of images of the jaws taken over subsequent days and weeks. Replacement rate was defined as the time taken for specific ground-down teeth to move one series forward in a labial direction.

Bite-grip tenacity tests

A servo-hydraulic materials testing machine (model 8872; Instron, Norwood, MA, USA) fitted with a 250 N load cell (Dynacell; Instron) was used to explore how variations in jaw and tooth morphology affect simulated bite tenacity. Fresh jaws from 16 rays (seven female, nine male) were tested using a custom-made rig. A 1.5 mm diameter hole was drilled through each of the lateral expansions of the lower jaw cartilages and the jaws were anchored to a rigid metal plate with nylon cable-ties. A pectoral fin was dissected free from a mature female *A. rostrata* specimen post mortem, and was clamped and connected by braided Kevlar line to the load cell via a pulley. The clamp rested on four ball bearings to provide low friction movement. The dissected fin was placed between the upper and lower jaws and gripped with an occlusal force of 0.98 N provided by a 100 g mass applied to the middle third of the upper jaw. A constant occlusal force was used as this experiment was designed to explore how variation in tooth form related to the force required to pull the pectoral fin out of the (simulated) bite. The 0.98 N occlusal force was used for pragmatic reasons related to the experimental set-up. Additionally, *in vivo* occlusal forces are not known for this species. However, given the rather gracile structure of the jaw cartilages and the suction-feeding nature of *A. rostrata*, it is likely that *in vivo* occlusal forces are relatively modest in this species. Computer-controlled actuator movement (1 mm s^{-1}) pulled the pectoral fin out of the jaws in an anterior (labial) direction, with the resisting 'pull-out force' recorded at 100 Hz on a computer. The distance of movement was standardised for all specimens, with the experiment ending immediately prior to the dissected fin achieving a 'pull-out' from within the occluded jaw. Frictional force intrinsic to the apparatus (<0.05 N) was determined by pulling the clamp–fin combination when not gripped by the jaws.

Statistical analysis

The relationship between TL and M_b was examined using regression analysis (SigmaPlot for Windows, version 11.0, Systat Software, Chicago, IL, USA). Linear regression analysis and one-way analysis of covariance (ANCOVA; PASW Statistics, SPSS, IBM, Armonk, NY, USA) were used to examine gender-related differences between tooth morphology, with TL added as the covariate. Data were visually assessed for the assumptions underlying ANCOVA, with a $\log_{10}(x)$ transformation substantially improving normality and heteroscedasticity. For tooth morphology parameters that varied in a non-linear fashion with TL, best-fit equations for curves were generated using Table Curve 2D software (Systat Software). Graphical representations were produced using SigmaPlot.

Two-way ANOVA with an all-pairwise Tukey test was used to determine differences in jaw morphometry for the factors sex and maturity stage (Table 4) (PASW Statistics). If necessary, data were normalised using $\log_{10}(x)$ or $\log_{10}(x+1)$ transformations prior to analysis.

One-way ANOVA was used to explore: (1) the differences between TW, TH and TSR, (2) the differences in mean peak pull-out forces among the maturity classes of *A. rostrata*, and (3) TBW_{lower}, TBW_{upper}, TR_{lower} and TR_{upper} from a random subset of animals. If significant differences arose, an all-pairwise Tukey *post hoc* test was employed to ascertain among-group variations.

At the locations T_{erupt}, $T_{occlusal}$ and T_{loss} (Fig. 9), TSR values at positions $P_{0\%}$, $P_{15\%}$, $P_{75\%}$ and $P_{100\%}$ were analysed for the following maturity stages: mature females (N=8), mature males (N=8), immature females (N=4) and

immature males (N=4). As t-tests demonstrated that mirrored positions on either side of the jaw were not significantly different (P<0.05), data were pooled for all corresponding positions. Two-way ANOVA with an all-pairwise Tukey *post hoc* test and a within-subjects factorial *post hoc* test was used to explore the differences in TSR for the factors maturity stage and position on the jaw (PASW Statistics).

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