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Reproductive cycle, nutrition and growth of captive blue spotted stingray, *Dasyatis kuhlii* (Dasyatidae)

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At Burgers' Ocean 7 male and 3 female blue spotted stingrays, Dasyatis kuhlii *were born over a period of 4.5 years. This paper describes the experiences of the captive breeding results of this species. The first two young died within 2 days of birth. One of them had an internal yolk sac, which may feed the young in the first few days. The other eight animals started to feed after 4 to 9 days on a variety of food types. Birth size of the young increased with increasing age of the parents. Mating occurred directly after parturition, so no seasonality could be defined. Gestation length ranged between 138 and 169 days, with a mean of 144.9 ± 9.0 days ($N = 11$). Litter size was one, possibly caused by only one active ovarium. Sexual maturity of the two parent animals is approximately 3.5 years. The average feeding rations for the adults ranged between 10.1% BW week*$^{-1}$ *(131 kcal kg BW*$^{-1}$ *week*$^{-1}$*) and 11.3% BW week*$^{-1}$ *(172 kcal kg BW*$^{-1}$ *week*$^{-1}$*), with a feeding frequency of 4 times per week. The relationship between body weight (BW) and wingspan (WS) is given as* $BW = 3.6 \times 10^{-5} * WS^{2.940}$ *(*$R^2 = 0.9645$*;* $N = 45$*).*

Keywords: reproductive cycle, nutrition, growth, captive blue spotted stingray, *Dasyatis kuhlii*

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INTRODUCTION

The blue spotted stingray, *Dasyatis kuhlii* (Müller & Henle, 1941) is a coastal batoid found in the waters of the Indo-West Pacific area (Allen, 2000) and Indian Ocean until Southern Africa (Compagno *et al.*, 1989). With a maximum wing span of 45 cm (Allen, 2000), this species is interesting for display in captivity. Captive breeding of elasmobranchs increased drastically in the last decennia due to increase of large public aquaria, increasing knowledge of water quality maintenance (e.g. Mohan & Aiken, 2004), nutrition (e.g. Janse *et al.*, 2004) and health. Henningsen *et al.* (2004b) describes successful reproduction of 99 elasmobranchs species in captivity, including 9 species of the genus *Dasyatis*. Captive breeding of the blue spotted stingray, *Dasyatis kuhlii* has not been described before. Breeding of elasmobranchs in captivity will help understand overall biological and husbandry knowledge of different species, and will decrease the necessity to get animals from the wild to be able to display elasmobranchs to the public.

Keeping animals in captivity through their life time can provide interesting information about species. The growth of an animal can easily be followed by measuring the body weight and/or size. Growth is directly related to the health status of the animal, the amount of captive stress, and most important the feeding practices. These include the feeding ration, frequency, type of food and vitamin additions. Nutrition practices of captive elasmobranchs vary enormously between different public aquaria (Janse, 2003) and species (Janse *et al.*, 2004). Mohan *et al.* (2004) describes both growth and nutrition for common captive sharks. Only one study describes in detail diet and food ration changes of a ray species, the spiny butterfly ray (*Gymnura altivela*) (Henningsen, 1996).

The first objective of this paper was to describe the breeding results of this species in captivity and provide information on the reproductive cycle, litter size and age of maturation. The growth characteristic of the young rays and description of the feeding strategy of the rays is the second goal of this study. The husbandry related issues may help to define management for other captive *Dasyatis* species.

MATERIALS AND METHODS

Animals

Since February 2000, a male and female were kept in the Lagoon display at Burgers' Ocean, Arnhem, The Netherlands. The animals originated from Indonesia. At arrival they had a wing span of 16 ± 1 cm. Based on the estimated relationship between body weight and age of the reared young rays born at Burgers' Ocean (see Results), the age of the rays at arrival was estimated to be 3 to 6 months. An estimated birth date of the female is 1 December 1999 ($T_{female} = 0$). Body weight (BW) was measured at an accuracy of 2 g and wingspan (WS) at an accuracy of 0.5 cm. The female died

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on day 1638. Necropsy showed only the left ovary to be active with different sizes of oocytes.

Aquarium

The Lagoon aquarium with a volume of 650,000 l artificial seawater is split in two parts. In one part (13 × 4 m) the rays are kept on a coral sand (ø = 2–6 mm) area surrounded by artificial rocks and artificial corals. This area consists of two horizontal plateaux at different water depths (3.0 and 1.2 m). Three acrylic windows on one long side show the aquarium to the public. Twenty-seven species of teleost fish (with a total number of fish of approximately 250) are in this area of the tank. Filtration of the whole aquarium consists of protein skimmer (70 m^3 h^{-1}) injected with ozone (0.03 mg ozone l^{-1} water), trickling filters (100 m^3 h^{-1}), sand filtration (15 m^3 h^{-1}) and for two years during this study a *Caulerpa rasemosa* refugium (10 m^2; flow of 0.4 m^3 m^{-2} h^{-1}). Water changes averaged 3% per month. Light above the aquarium consists of a combination of HQI and fluorescent lamps (14L:10D). A dawn and dusk period is created by switching all lights on or off in 5 stages over 1 hour period.

Water temperature at the lagoon tank is kept at 25.0 ± 0.5°C, salinity at 33.0 ± 0.3‰ and pH at 8.0–8.1. Other water quality parameters are: <0.01 mg $NH_4^+{-}N\,l^{-1}$, <0.01 mg $NO_2^-{-}N\,l^{-1}$, <35 mg $NO_3^-{-}N\,l^{-1}$ and <0.8 mg $PO_4^{3-}{-}P\,l^{-1}$. Water quality of the quarantine system was the same as the lagoon tank concerning temperature, salinity, pH, ammonia and nitrite. Nitrate and phosphate levels were lower at <3.0 mg $NO_3^-{-}N\,l^{-1}$ and <0.5 mg $PO_4^{3-}{-}P\,l^{-1}$.

The young were transferred directly after birth to a quarantine tank (120 × 60 × 50 cm), with water from the original Lagoon aquarium. Over a period of one week the water was renewed by water from the quarantine system, to prevent any chemical stress. The quarantine tank is part of a 6000 l system with UV, protein skimmers and trickling tower as filtration. On the bottom of the tank fine coral sand (ø = 2–4 mm) is placed so the animal can bury itself, which happens for more than 90% of the time. When young were around 6 months old they were transferred to a 120 × 120 × 50 cm tank, followed by a 200 × 200 × 70 cm tank at an age of 1 year.

Nutrition

The adult rays were always fed individually from a stick, making it known how much they eat per feeding. When rays became mature they were fed 4 times per week. Every feeding day another type of food was fed consisting of mackerel, anchovy, gamba or whiting (Table 1). Rarely one of the four food types was replaced by squid, scad or herring. The young rays were fed individually from a stick with small pieces of the food types described for the adults with additional mussel (*Mytilus edulis*) and razor shell (*Ensis siliqua*). Vitamin addition was done once per week inserted in the food as tablet (Twilmij) to adults or liquid (Eurovet) form to juveniles to the body weight of approximately 500 g (Table 2). About 30% of the time the animal discarded the tablet.

Feeding ration (Table 4) for the adults was calculated over a 6 weeks interval, of which 4 weeks before the measurement of the animals and 2 weeks after.

Table 1. Type of food and energy content (kcal/g) given to captive *Dasyatis kuhlii.*

		kcal g^{-1}
Whiting	*Merlangius merlangus*	1.102
Mackerel	*Scomber scombrus*	2.466
Atlantic herring	*Clupea harengus*	1.628
Gamba	*Penaeus* sp.	1.111
Squid	*Loligo* sp.	1.580
Scad	*Trachurus trachurus*	1.063
Anchovy	*Engraulis encrasicolus*	1.201
Mussel	*Mytilus edulis*	–

The wingspan (WS) and body weight (BW) relationship was determined using the allometric equation: $BW = a\ WS^b$.

Percentage specific growth rate (%SGR) is calculated as the change in body weight (BW) over time (t) as (De Silva & Anderson, 1995):

$$\%SGR = \frac{(\ln(BW_{t_2}) - \ln(BW_{t_1}))}{(t_2 - t_1)} \times 100$$

where BW_{t1} is the initial weight at time t_1 or BW_{t2} is mean weight at time t_2.

RESULTS

Breeding results

Seven male and three female stingrays have been born (Table 3), of which the first two died within two days after birth. These two animals died with skin haemorrhages possibly caused by aggression of other fish in the Lagoon aquarium. This may have caused death or the animals were too weak after birth. At necropsy, histology showed no abnormalities and bacteriology was negative on both animals. An internal yolk sac of 15 × 10 mm was found in the visceral cavity of the second ray. Histology showed an open vacuole tissue.

Table 2. Vitamin and mineral addition to the food of captive *Dasyatis kuhlii.*

	Dosage juvenile ($kg\ BW^{-1}\ wk^{-1}$)*	**Dosage adult ($kg\ BW^{-1}\ wk^{-1}$)****
Thiamine	5.0 mg	59.4 mg
Riboflavin	4.0 mg	1.2 mg
Nicotinic acid	25.0 mg	2.4 mg
Panthothenic acid	6.0 mg	1.2 mg
Pyridoxine	2.5 mg	0.7 mg
Biotin	–	23.7 μg
Folic acid	–	11.9 μg
Cyanocobalamin	0.6 μg	2.4 μg
Ascorbic acid	4.0 mg	29.7 mg
Vitamin A	100.000 i.u.	2968 i.u.
Vitamin D3	50.000 i.u.	95 i.u.
Vitamin E	8.0 mg	11.9 mg
Iodine	–	35.6 μg

*at BW = 0.15 kg, **at BW = 1.05 kg; for the adults in the last six months.

Table 3. Birth data and reproductive cycle of captive *Dasyatis kuhlii.*

No.	Time of birth (d)	Gender	Appearance of bite marks (d)	Appearance of bite marks after parturition (d)	Recorded parturition interval (d)	Gestation length (d)	Body weight at birth (g)
1	0*	M	Unknown	–	–	–	–
2	138	M	Unknown	–	138	138	–
			279	–		141	–
3	425	M	Unknown	–	287	146	–
			594	–		169	–
4	746	M	747	1	321	152	–
5	884	M	884	0	138	138	–
6	1025	F	1026	1	141	141	–
7	1170	M	1170	0	145	145	74
8	1310	F	1309	−1	141	141	89
9	1453	F	1447	−7	143	143	86
10	1593	M	1585	−8	140	140	91

*t = 0 equals 19 January 2003.

The recorded parturition interval is average 177.1 days (±72.5), with a range of 138–321 days (Table 3). The recorded parturition interval for ray numbers 2, 5, 6, 7, 8, 9 and 10 was average 140.9 days (standard deviation of 2.5 days); ray numbers 3 and 4 show a gestation period more than twice as long (287 and 321 respectively).

Until 7 days before birth the neonate is visibly moving within the mother's body. The female sits in the last week regularly on the distal tips of her pectoral fins and the tips of her pelvic fins forming a tunnel. All young were found in the early morning buried under sand.

Mating happened directly after giving birth to the young, since different biting marks were seen on the dorsal surface of the female in the days after parturition (Table 3). The mating has never been seen. The appearance of the bite marks were seen 8 days before to the day after the birth of a young. The comparisons of recorded parturition interval and date of appearance of dermal wounds would suggest two neonates have been missed and eaten by other fish or disappeared in the artificial surrounding, resulting in two new gestation lengths. The range of the gestation length would then be: 138–169 days (Table 3), with an average of 144.9 ± 9.0 days (N = 11).

Nutrition and growth

The young started to eat 4 to 11 days after birth, with an average of 7.5 ± 2.5 days (N = 8). The type of food offered varied every day. The type of food eaten for the first time also varied between gamba (N = 2), mussel (N = 1), mackerel (N = 1), scad (N = 2), squid (N = 1) and krill (N = 1). The young are fed 6 times a week. The adults were fed 4 times per week. Figure 1 gives an overview of the change in feeding ration over time, where the moments of birth are described in the figure. No significant correlation could be detected between parturition and food intake.

Table 4 gives an overview of the feeding ration of the adult female and male. On the day of parturition the female did not

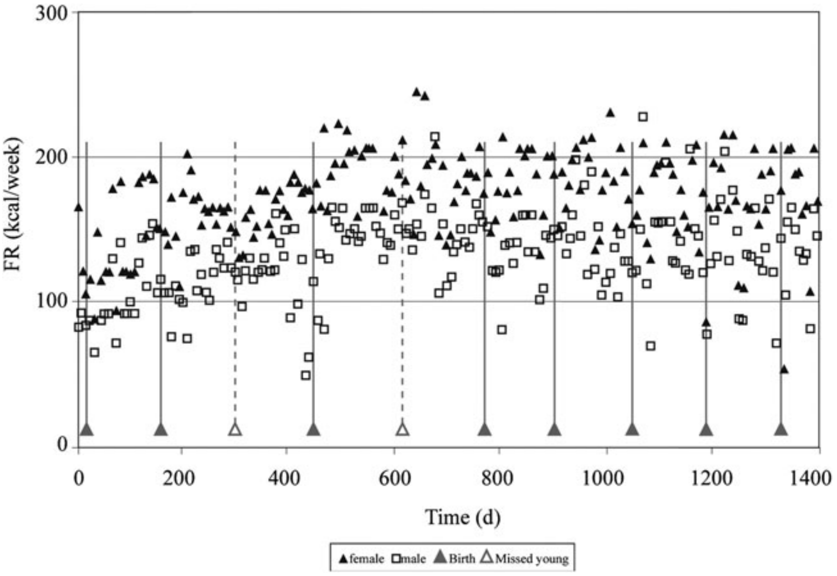

Fig. 1. Changes in feeding ration (FR) over time in captive *Dasyatis kuhlii.*

Table 4. Body weight, wingspan and feeding ration of adult captive *Dasyatis kuhlii*.

	Time* (d)	Measured body width (cm)	Measured body weight (g)	Feeding ration			
				(kcal wk^{-1})	(kcal kg BW^{-1} wk^{-1})	(g wk^{-1})	(% BW wk^{-1})
Male	1033	30.0	910	128	135	102	10.9
	1396	31.0	1100	145	131	120	10.9
Female	1033	32.0	1053	176	172	119	11.3
	1396	32.0	1200	172	143	121	10.1

*t = 0 equals 19 January 2003.

eat during 3 cases, 6 times she ate normally or a little less, and one day she was not fed.

The wingspan at birth (WS_{birth}) increased with increasing age of the mother animal (T_{female}) (Figure 2) ($WS = 0.9629*T^{0.3360}$; $R^2 = 0.9113$; N = 10). Figures on weight at birth were limited (Table 3).

On a regular basis the body weight and wingspan was measured of the animals. Figure 3 gives the relationship between body weight (BW) and age of the captive born animals, with the statistical analysis in Table 5.

The relationship between wingspan (WS) and age of the captive born animals is given in Figure 4, with the statistical analysis in Table 6.

Combining all figures from the two adult and eight young animals a relationship between body weight (BW) and wing span (WS) can be given in Figure 5 and can be expressed as $BW = 3.6 \times 10^{-5} * WS^{2.940}$ ($R^2 = 0.9645$; N = 45).

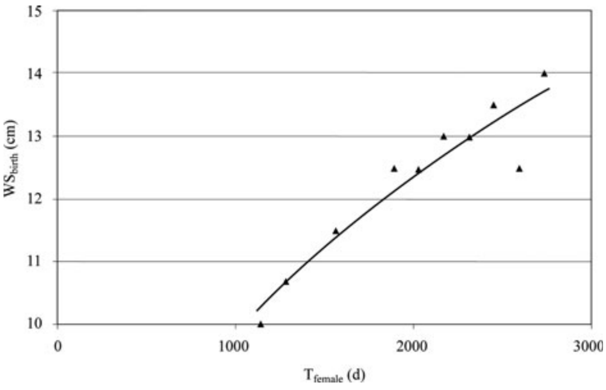

Fig. 2. Relationship between wingspan at birth (WS_{birth}) and age of the mother (T_{female}).

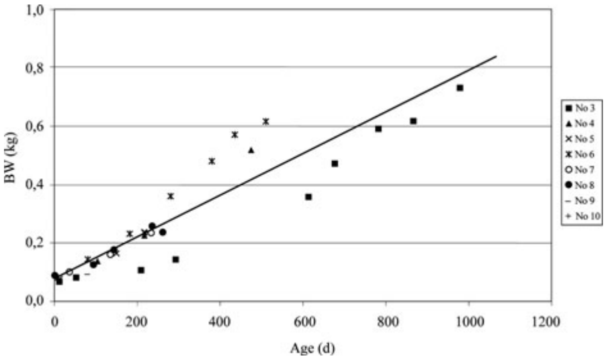

Fig. 3. Relationship between body weight (BW) and age for captive *Dasyatis kuhlii*.

The relationship between the specific growth rate (SGR) and age (t in years) is expressed as SGR = −1.929Ln(t) + 14.503 ($R^2 = 0.6306$; N = 30) (Figure 6).

Table 5. Linear regression between body weight and age.

Animal No.	Intercept	Regression coefficient	R^2	N
3	0.0017	0.0007	0.9627	11
4	0.0437	0.0009	0.9867	4
5	0.0623	0.0008	0.9888	3
6	0.0531	0.0011	0.9933	8
7	0.0007	0.0007	0.9986	6
8	0.0007	0.0007	0.9502	5
9	0.086	0.00004	1	2
10				1
Total	0.0851	0.0007	0.8764	41

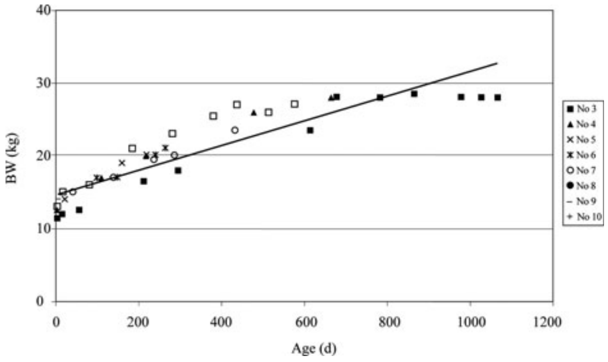

Fig. 4. Relationship between wingspan (WS) and age for captive *Dasyatis kuhlii*.

Table 6. Linear regression between wingspan and age.

Animal No.	Intercept	Regression coefficient	R^2	N
3	12.645	0.0169	0.9335	12
4	14.030	0.0220	0.9590	5
5	13.04	0.0344	0.9723	4
6	14.822	0.0246	0.9248	9
7	13.684	0.0232	0.9860	6
8	13.698	0.0274	0.9702	5
9	12.5	0.0438	1	2
10				1
Total	14.203	0.0178	0.7525	44

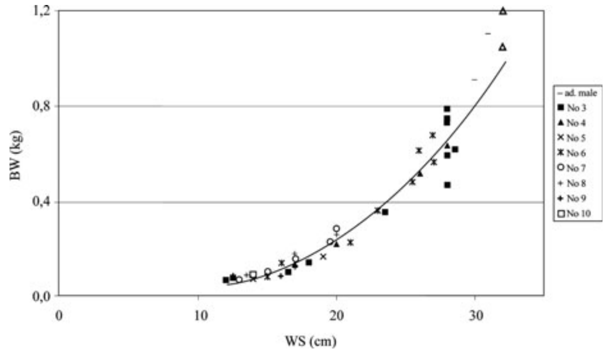

Fig. 5. Relationship between body weight (BW) and wing span (WS) for captive *Dasyatis kuhlii*.

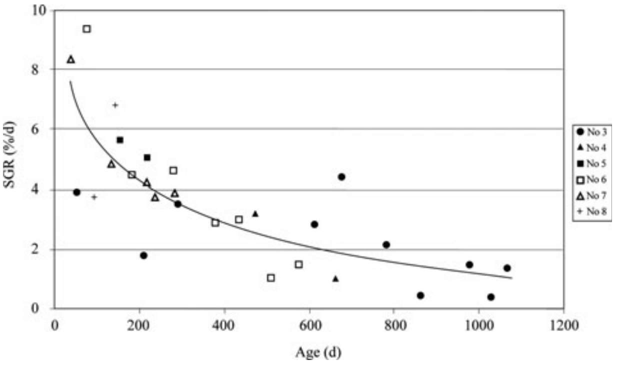

Fig. 6. Relationship between specific growth rate (SGR) and age of six captive born *Dasyatis kuhlii*.

DISCUSSION

The recorded parturition interval ranged between 138 and 321 days. Since mating is directly after parturition this period could give an indication of the gestation time of *D. kuhlii*. The large variation may be caused by an over-gestation time earlier described as 20% in cownose rays (*Rhinoptera bonasus*) until 100% for yellow stingrays (*Urobatis jamaicensis*) (Henningsen *et al.*, 2004a). A smaller variation of the gestation time is described in captive *D. americana* ranging between 135 and 226 days (Henningsen, 2000).

The occurrence of dermal wounds in *D. sabina* is described as a useful non-invasive technique to determine reproductive activity (Kajiura *et al.*, 2000). Bite markings with *D. kuhlii* were mostly defined on the dorsal side of the pectoral fins and especially on the caudal ridge or outer tip of the pectoral fin. The same has been observed in *Urolophus halleri* and *U. aurantiacus* where the male bit the pectoral fin edges of the female (Nordell, 1994). Combining the recorded parturition interval and date of appearance of dermal wounds will result in a gestation length between 138 and 169 days (Table 3), with an average of 144.9 ± 9.0 days.

Mating occurred in *Dasyatis kuhlii* directly after parturition, which indicates no breeding season. In a survey on a wild population of *D. kuhlii* in Tanzania of a total of 117 females caught throughout the year pregnant females were recorded in November (N = 1), December (N = 4), January (N = 2), April (N = 1) and July (N = 1) (Darracott, 1977). This would indicate a breeding season. December and January show the largest number of gravid females which in Tanzania is the turn of monsoon. The constant availability of food and constant abiotic circumstances in the captive environment may result in the lack of a breeding season in captivity. Observations on *Dasyatis americana* showed mating after parturition *in situ* (Chapman *et al.*, 2003) and *ex situ* (Henningsen, 2000). This supports the fact that *ex situ* observations on the reproductive cycle in *D. kuhlii* may be copied to the wild. Also the temperate oviparous thorny skate (*Amblyraja radiata*) has a reproductive cycle that is continuous throughout the year (Sulikowski *et al.*, 2005), while other batoids show an annual reproductive cycle *Dasyatis pastinaca* (Ismen, 2003), *Dasyatis sabina* (Snelson *et al.*, 1988; Tricas *et al.*, 2000), *Dasyatis say* (Snelson *et al.*, 1989) and the eastern shovelnose ray, *Aptychotrema rostrata* (Kyne & Bennett, 2002).

At the age of 4 years, the female gave birth to the first young. With a development period of 146 days, this would suggest the male and female were sexually mature at an age of 3.5 years. This figure can only be used as indication for other *D. kuhlii* since it is not known if the growth of the animals was the same as in the wild. Also they may have been mature earlier than 3.5 years, but did not result in successful mating or embryo development. Also there may be a difference between sexual maturities of both sexes. This has been described within an *in situ* population of blue stingray *Dasyatis chrysonota chrysonota*, where the estimated age at first maturity for males was defined at five years and seven years for females (Cowley, 1997). Also males mature at a smaller size than females for *Dasyatis pastinaca* (Ismen, 2003).

The minimum disc width of pregnant females *in situ* was 30 cm (Darracott, 1977).

This study shows a litter size of one for *D. kuhlii*, with an average gestation time of 145 days; this equals a fecundity of 2.5 young per year per female. The latest measured wing span of the female was 32 cm, while the maximum body width varies between 40 cm (Lieske & Myers, 1994) and 45 cm (Allen, 2000). Since the female can grow bigger the litter size may increase, however it is not expected the number of young will increase drastically since the size of the young still increases with the size of the female. In captive *D. americana* the litter size ranged from 2–10 and varied directly with maternal size and indirectly with mean size and weight of neonate (Henningsen, 2000). In the eastern shovelnose ray, *Aptychotrema rostrata* a positive linear relationship between maternal total length and uterine fecundity is described (Kyne & Bennett, 2002). The female in this study showed after death only to have one active ovary. It is unknown if this is normal for this species, or that both ovaries should be active and thus more young could be produced in one litter. Many elasmobranch species have two active ovaries. The development of only one ovary is also described in *Carcharhinus brevipinna* (Joung *et al.*, 2005) and *Carcharhinus dussumieri* (Teshima & Mizue, 1972). While in *Rhinoptera bonasus* no uterine or ovary development has been observed in the right reproductive tract and only one young was found in the left tract (Neer & Thompson, 2005). The above studies did not provide a hypothesis for why only one ovary develops. One hypothesis of this anomaly within *D. kuhlii* is individual differences within this species, though the study of Neer & Thompson (2005) showed 100% occurrence in the population of *R. bonasus*. So this may also account for *D. kuhlii*. Another hypothesis is the development of only one reproductive tract will occur in species with only a few young. This way the young can grow larger. Such low birth rate might be considered disadvantageous in terms of maintaining levels of recruitment, though this might be compensated for by the relatively large size at birth, since larger neonates are presumably less likely to be preyed on.

A second goal of this study was to describe the growth of the young and the nutrition of the captive rays.

Growth in elasmobranchs is often characterized by the Von Bertalanffy growth function (VBGF), where the change in weight or length over time is expressed as a function of the asymptotic (maximum) weight or length. The data in this study are mostly concentrating on young animals, which make it impossible to have an asymptotic weight figure. No data are found in literature on the specific growth rate on elasmobranchs. It is added in this study as a descriptive purpose, even though there is a wide variation found in the specific growth rate.

Only one study was found to give the length (L)/weight (W) relationship of *in situ D. kuhlii* (Darracott, 1977). Of all animals ($N = 351$) the relationship was expressed as $W = -2{,}73L^{1{,}88}$, for females ($N = 117$) $W = -2{,}90L^{2.0}$ and males ($N = 85$) $W = -2.39L^{1.65}$. These figures cannot be compared with the captive population of this study since the length of the animals was used instead of the wing span. Since rays often lose part of the tail it is better to use wing span as size indication. However, from these figures it can be concluded that the animals have an allometric growth and size differences between the male and female. Differences in modal width and weight of the population can be seen between male and female fish, the females being wider and heavier (female mode 35–40 cm, 1.25–1.5 kg; male mode 30–35 cm, 1.0–1.25 kg) (Darracott, 1977). The figures from this study are over too short a period to give conclusive difference between the sexes. The relationship between body weight and wing span is a practical tool when working with captive *D. kuhlii*. When estimating the width of the animal, an estimation can be made using this graph of the body weight. This can be used to calculate a food ration as a percentage or ratio of the body weight.

The feeding ration of the parents ranged between 10.1 and 11.3% BW week^{-1}. These figures are twice as high, than the 4–6% BW week^{-1} advised for bottom dwelling rays like Dasyatidae (Janse *et al.*, 2004). In captive *Dasyatis violacea* when consuming squid (*Loligo* sp.) the feeding ration decreased from 6–7% BW d^{-1} for juveniles to 1.3% BW d^{-1} for adults (Mollet *et al.*, 2002). When feeding every day this would mean 9.1% BW week^{-1}, which is a little less than was fed to the smaller *D. kuhlii* in this study.

The feeding ration of spiny butterfly ray (*Gymnura altavela*) increased from 4.2–8.8 % BW week^{-1} in their captive life (Henningsen, 1996). This increase was caused due to acclimation problems in the beginning.

To include the difference of food items offered, feeding ration can better be expressed as a function of the energy content of the food (Janse, 2003; Janse *et al.*, 2004). The adult *D. kuhlii* were fed at a range of 131 and 172 kcal kg BW^{-1} week^{-1}. These figures are much higher than for adult captive *Dasyatis violacea* that were fed at 9.1% BW week^{-1} on squid with a wet-mass energy value of 0.800 kcal/g for squid (*Loligo opalescens*) (Mollet *et al.*, 2002). From this it can be calculated that the adults were fed at 73 kcal kg BW^{-1} week^{-1}. Another study on captive sharks like, *Carcharhinus plumbeus* (ranging between 32 and 112 kcal kg BW^{-1} week^{-1}) (Janse, 2003) was also lower than the ration for the rays in this study. However, this RAM ventilating shark has a much higher activity and makes comparison difficult with an inactive ray such as *D. kuhlii*. Figures from other species of bottom dwelling sharks or rays expressed in energy content of the food are not available in literature. It is advised to express feeding ration in future studies both as % BW week^{-1} and kcal kg BW^{-1} week^{-1}. Still the feeding strategy as feeding ration and food type used in this study proved to be successful for these animals when looking at the breeding success.

The nutrition of the female adult does not change within the reproductive cycle (Figure 1). Liver reserves are probably stored and metabolized continuously throughout the year. The same has been described in the oviparous thorny skate (*Amblyraja radiata*), where the hepato-somatic index shows no significant changes in female thorny skates over the reproductive cycle (Sulikowski *et al.*, 2005). In contrast Mayer (1984) mentioned an increase of food intake one month prior to parturition with captive *D. americana*. This is also described for an oviparous shark, *Scyliorhinus canicula*, which displayed seasonal variations in liver mass as a result of lipid deposition occurring during different times of the reproductive cycle (Craik, 1978), though this species has a seasonal reproductive cycle (Henderson & Casey, 2001) that may effect the nutrient storage.

Nutrition in the first days of the newly born rays is done via the internal yolk sac and reserves stored in the liver. The internal yolk sac is absorbed yolk material originating from the external yolk sac. This phenomenon has also been described for the small spotted dogfish, *Scyliorhinus canicula*, an oviparous species (Wrisez *et al.*, 1993) and the ovoviparous Squalidae, *Centrophorus* cf. *uyato* (McLaughlin & Morrissey, 2005) and *Centrophorus granulosus* (Guallart & Vicent, 2001). Prior to hatching the yolk transfers from the external yolk sac into the spiral gut, where its digestion starts, and will be ended only one week after birth. Since the gut cannot accommodate all the yolk and the external yolk sac has to be resorbed completely before birth, an internal yolk sac develops, playing the role of a transient storage organ within the abdominal cavity (Wrisez *et al.*, 1993). All neonates of *D. kuhlii* had no external yolk sac at birth. To cover the first few days the rays have absorbed the internal yolk material. This explains why the last six young started to eat between the 4th and 11th day of their life on different types of food (Table 4). The first two animals never ate. There seemed to be no preference for the type of food since different types were offered. Young captive *D. americana* ate shrimp and scallop, though no specific preference was described (Mayer, 1984). In *D. pastinica* a shift in the diet occurred with increasing body length, from crustaceans to teleosts (Isman, 2003). Analysis of stomach content of *Dasyatis chrysonota* showed a diet shift from primarily a mollusc (*Donax* sp.) in medium sized animals to a crustacean (*Callinassa* sp.) at adult size (Ebert & Cowley, 2003). The diet of adult *D. kuhlii* in the wild consists of sand dwelling invertebrates (Lieske & Myers, 1994), like polychaete worms (Darracott, 1977) and crustaceans (Darracott, 1977; Compagno *et al.*, 1989). Within this study the animals are fed a combination of teleosts (75%) and crustaceans (25%). Even though this diet is not the same as in the wild, the nutritional values are similar and thus can act as a good substitute. However, feeding of captive elasmobranchs is mostly done with pre-frozen food, to eliminate the possibility of parasitic infection and ensure the continuity of food availability. Loss of vitamins and minerals due to food transport, freezing, storage, and preparation make vitamin and mineral supplementation necessary (Janse *et al.*, 2004).

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