The occurrence and development of 'Siamese twins' and other abnormalities in *Oreochromis mossambicus* (Pisces, Cichlidae)

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Specimens from a clutch of *Oreochromis mossambicus* (Pisces, Cichlidae) exhibited a number of abnormalities such as enlarged pericardia, thin tube-like hearts, large depressions in the yolksac, spinal deformities and twinning. Descriptions of the differences in early ontogeny of two pairs of 'Siamese twins' over a period of at least eight days are given. The developmental pattern of the twins is similar to that of normal specimens but the developmental rate is slower in the twins. Possible explanations for these occurrences of polyembryony are given.

Daar is gevind dat 'n broeisel van *Oreochromis mossambicus* embrio's abnormaliteite getoon het, soos byvoorbeeld vergrote hartsakke, dun pypvormige harte, groot holtes in die dooiersak, misvormings van die ruggraat en tweelingvorming. Beskrywings van die verskille in die vroeë ontwikkeling van twee pare Siamese tweelinge gedurende 'n periode van nie minder nie as agt dae word verskaf. Die ontwikkelingspatroon van die tweelinge is gelyk aan die van 'n normale embrio maar die ontwikkelingstempo is stadiger in die tweelinge. Moontlike verklarings vir hierdie voorkoms van poli-embrionie word voorgestel.

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Incidents of 'twins' in fishes have been reported in *Salmo salar* (Bruno 1990), trout, sharks, *Poecilia* sp., *Pseudocrenilabrus multicolor* (Reichenbach-Klinke 1972, Reichenbach-Klinke & Elkan 1972) and batrachoidids (Stephens 1973), as well as in *Oreochromis mossambicus* and a hybrid cross between *Oreochromis urolepsis hornorum* × *Oreochromis niloticus* (Hulata & Rothbard 1978). Other species of cichlids exhibiting this phenomenon are *O. niloticus*, *Oreochromis aurea* and the Taiwanese red tilapia (Huang, Cheng, Chang, Chao & Liao 1987). In twinning, a common yolksac is shared by the twins which may be relatively normal otherwise. In many instances the twins are cojoined by other body parts such as the head or the tail, or one individual appears normal with a malformed sibling attached on its flank or ventrum. The above authors briefly comment on these abnormal occurrences. In this paper, the development of two pairs of twins in the mouthbrooding cichlid *O. mossambicus* was followed over a period of at least 8,5 days and was compared to the development of normal siblings from the same clutch. The development of one set of the twins was followed beyond the embryonic and into the juvenile period. Brief mention is made of other abnormal specimens.

Materials and methods

The embryos were incubated in a separation funnel within an aquarium. Water temperatures ranged from 25 to 27°C. In order to simulate churning in the buccal cavity of the adult female, water flowed from the bottom to the top of the funnel. The embryos were periodically removed from the incubator and placed under a stereo microscope where drawings were made and notes taken. When possible photomicrographs were also taken. Afterwards the embryos were returned to the incubator. The twins that reached the juvenile period were kept in a normal aquarium. The figures do not show all the anatomical structures described in the text owing to the activity of the specimens during microscopic examination. Because repeated observations were done over time, the use of an anaesthetic to curtail movement was not possible.

The exact time of fertilization was not known. When these abnormalities were first noted the embryos were between 2 d 4 h and 2 d 19 h old. To determine which end of this 15 h discrepancy was most appropriate, the level of development of the specimens from this study was compared to that of the same species as described by Holden & Bruton (1992). As the level of development of the embryos under discussion was more advanced than the development of known-aged individuals at 2 d 19 h, it was assumed that this age was more realistic and probable. For ageing purposes in this study, this upper limit was used. Terminology for anatomical structures is taken from Holden & Bruton (1992).

Results

One set of twins was relatively normal in its characteristics except that one of the individuals had a deformed tail which curled anteriorly at the interface between the caudal peduncle and the caudal fin. In the second pair of twins, one individual was normal but the other was extremely malformed. Throughout this paper, the terms 'normal twins' and 'deformed twins' will be used to describe the first and the second pair, respectively.

Developmental descriptions

Age 2 d 19 h–6 d 19 h

Deformed twins and normal specimens: The one twin which was normal in its development will be referred to here as B and the malformed individual as A. The twins were situated at opposite sides of the yolksac, as in the normal twins (Figures 1 & 2). Twin A had a head, body and tail but most

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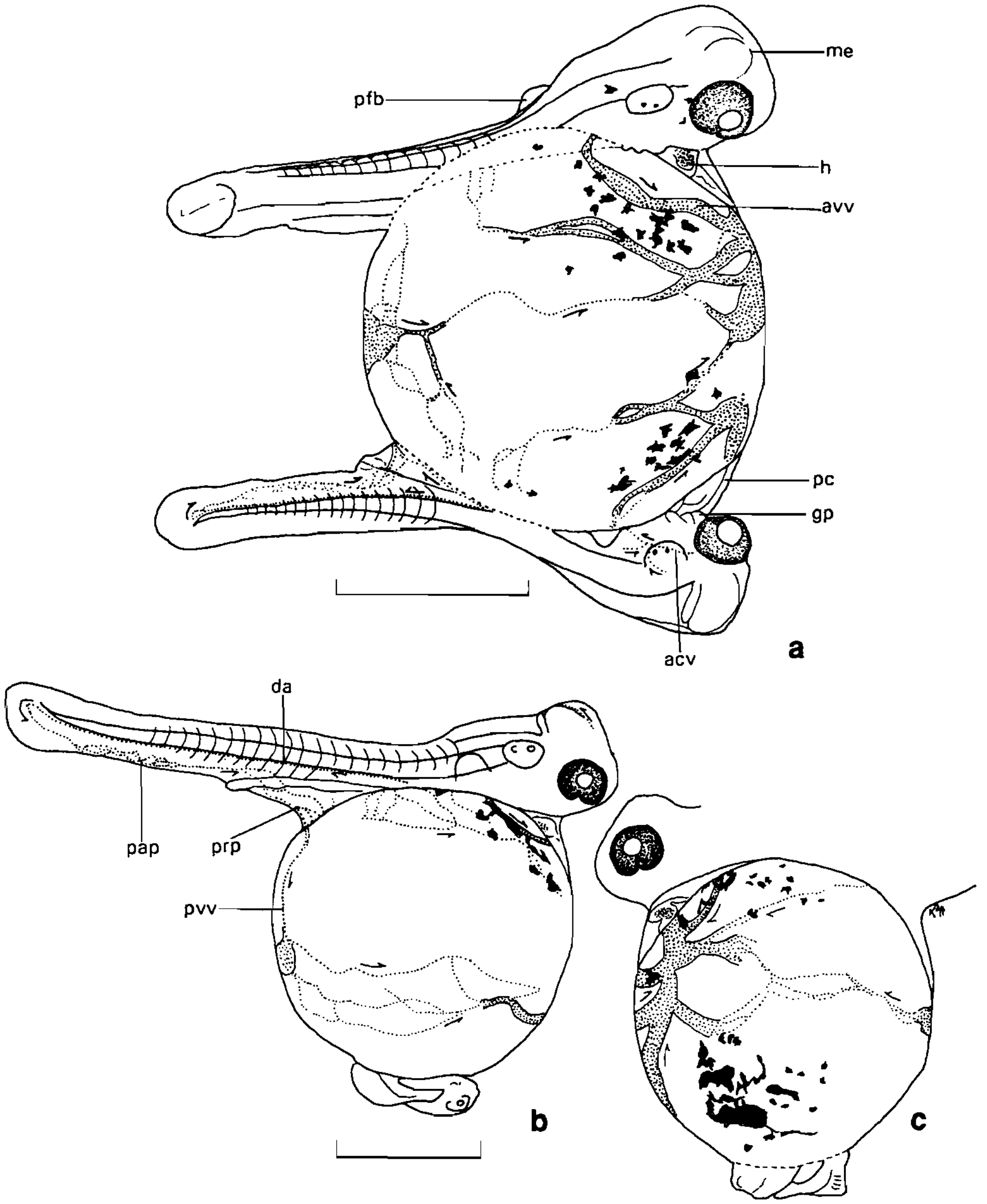

Figure 1 Lateral views of normal (a) and deformed (b & c) twins of *O. mossambicus* at age 3 d 16 h. Arrows indicate the direction of blood flow (acv = anterior cardinal vein, avv = anterior vitelline vein, da = dorsal artery, gp = gill pouches, h = heart, me = mesencephalon, pap = postanal respiratory plexus, pc = pericardial cavity, pfb = pectoral fin bud, prp = preanal respiratory plexus, pvv = posterior vitelline vein). Scales = 1,0 mm.

of the anatomical features were not distinguishable as individual structures. The tail curved latero-anteriorly such that its tip lay just posterior to the head. Some muscular contractions were noted in the tail. There was no heartbeat or circulation and no lenses were present in the eyes. The level of development in Twin B was slightly more advanced than in the normal twins. The yolksac pigmentation in Twin B resembled that of the normal twins, but extended more towards the central yolksac below Twin A. Major differences occurred in the vitelline plexus. In Twin B the vessels of the vitelline plexus followed the same route as in the normal twins, except that several large vessels coalesced to form one sheet-like flow into the heart-tube. There were no vitelline veins leading to Twin A (see Figures 1b & c). The mean heartrate for Twin B at age 3 d was 163 beats/min while the mean heartrate of the normal twins was 110 and 103 beats/min (Table 1).

After 24 h there were two vascular networks along the lateral flanks of the yolksac but the blood flowed only to Twin B. There was no eye pigmentation in Twin A, as recorded for all the other twins (Figures 1b, c & 2b).

By age 4 d 21 h pigmentation was present in the eyes but not in the lenses of Twin A. Otoliths had formed but there was still no heartbeat or distinguishable heart-tube. The level of development of Twin B was slightly in advance of the normal twins but less developed than that of the normal specimens from the clutch. The most obvious differences between Twin B and the normal twins was in the presence of caudal fin rays with corresponding radial loops, and in the gill pouches; these structures were evident in the normal twins after an additional 24 h. In the normal specimens the profundal caudal vein had formed and emptied into the

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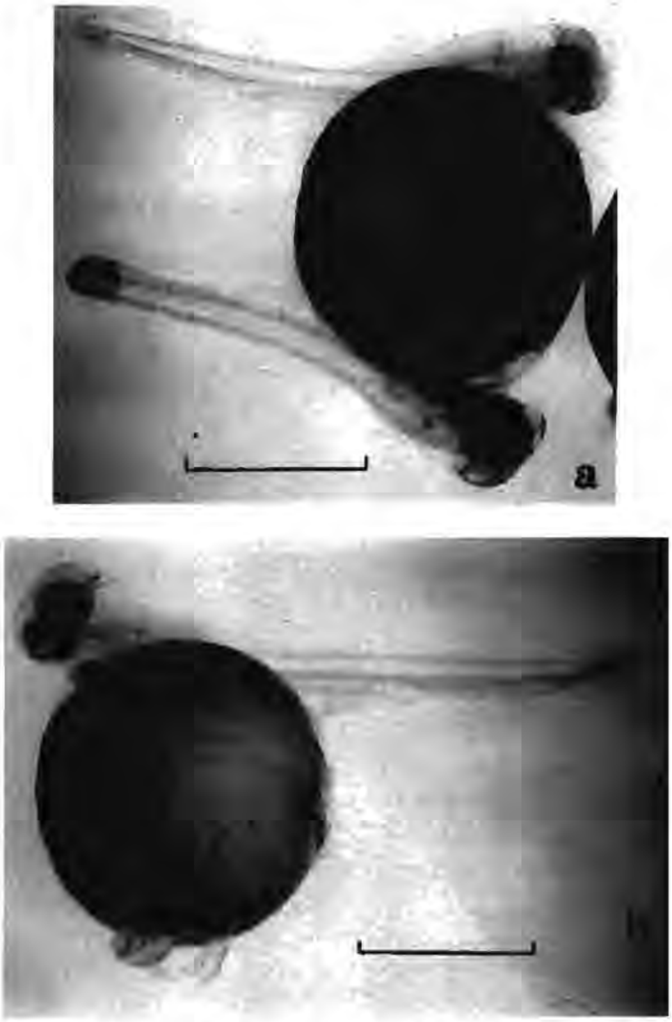

Figure 2 Photographs of normal (a) and deformed (b) twins of *O. mossambicus* at age 3 d 16 h. Scales = 1,0 mm.

Table 1 Mean heartrate (beats/min) for normal and deformed twin embryos and normal clutch embryos of *O. mossambicus* at different ages

Age (days/ hours)	Heartrate (mean beats/min)				
	Normal twins		Deformed twins		Normal specimens
	A	B	A	B	
2 d 19 h	123	125			
3 d	110	103		163	
3 d 1 h	185	179			
3 d 15 h	125	125		123	
4 d 21 h	126	133		134*	134
4 d 23 h					150
5 d 18 h	136	142		128	134, 134, 146
6 d 19 h	123	124	88	123	119, 113
7 d 20 h	108	108*		104*	
8 d 17 h	122	118		104*	115

* These values indicate an erratic heartrate whereby the range of the readings was greater than 10 beats/min

posterior cardinal vein. There was a reduction in the inferior caudal vein in preparation for its replacement by the profundal caudal vein. In addition to radial loops in the caudal finfold, there was another dorsal loop, the urostylar artery and vein. The segmental veins and arteries were more complex, the atrium had begun to migrate dorsal to the ventricle and circulation had begun in the gill arches and the newly formed gill filaments. The vitelline plexus was more extensive and symmetrical, covering the entire yolksac surface. The hepatic and intestinal vitelline networks were present. A yellow substance was present in the lumen of the gastro-intestinal tract. Most of these features were not evident in the normal twins until another 21 h at age 5 d 18 h.

By age 5 d 18 h the deformed Twin B was slightly less advanced in its development compared to the normal specimens of similar age. The postanal plexus was gone, the four components of the heart were distinguishable and the gut lumen was open at the vent (Figures 3a & b). Twin A had a rounded snout and pectoral fins had developed but were reduced in size compared to all other specimens. The pericardium was bloated and some pulsating activity was noted. However, no heart nor blood circulation was visible within the pericardial cavity. A red spot had developed between the body and the yolksac interface. The yolksac was sunken in below the head and blood flowed towards the pericardium but was not seen to enter the pericardial cavity. Yolksac absorption was uneven. Twin A lay closer to the head and ventral to the pericardium of Twin B.

By age 6 d 19 h the snout of Twin A was long and rounded, resembling the shape of a duck's beak. There were some iridocytes above the swimbladder and the visceral cavity. Otherwise there was no body pigmentation except for the eyes, which were black. There was no visible circulation but blood pools were present inside the head between the gills and opercular areas. Pulsations in the pericardium were erratic at about 88 beats/min. On occasion the tail and body twitched.

Twin B was still only slightly less advanced in its level of development compared to normal specimens of similar age. There were iridocytes in the eyes, on the operculum and on the body above the visceral cavity. Pigmentation and colouration was slightly more extensive than that described for the normal twins at this age. There were 16 caudal fin rays, at least six of which were segmented. All but the peripheral rays had radial loops and some mesenchymal rays were present in the posterior of the dorsal fin. The pectoral fins were moving. As with the normal twins, the lumen of the gastro-intestinal tract was full of a yellow substance. The edges of the pericardium of Twins A and B were touching owing to the movement of A towards B as a result of yolk absorption.

The normal specimens differed from Twin B in that the yellow colouration and iridescence were more extensive, and they were more advanced in dorsal, anal and pectoral fin ray development. The finfolds were also more differentiated. These embryos were also more active and the extrusion of faeces indicated that the intestinal tract was functional and that first exogenous feeding had begun.

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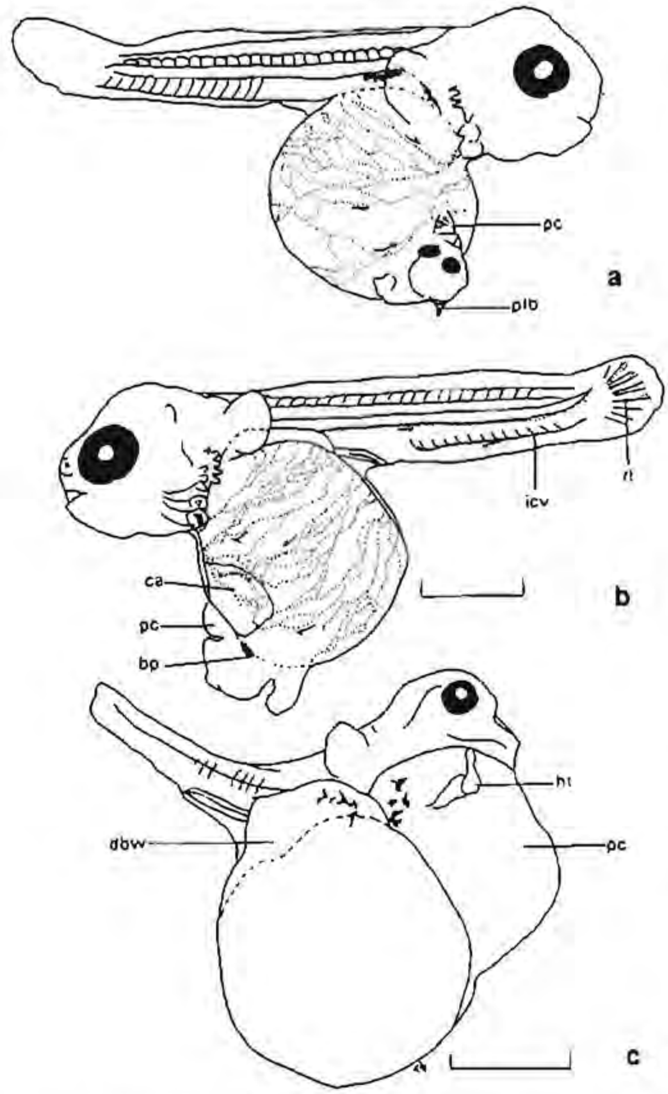

Figure 3 Deformed twins of *O. mossambicus* (a & b) and a specimen showing other abnormalities (c) at age 5 d 18 h. In c, note the bloated pericardial cavity and the distortion of the mouth, heart and descending body wall over the yolksac (bp = blood pool, ca = cavernous area, dbw = descending body wall, ht = heart-tube, icv = inferior caudal vein, pc = pericardial cavity, pfb = pectoral fin bud, rl = radial loop). Scales = 1,0 mm.

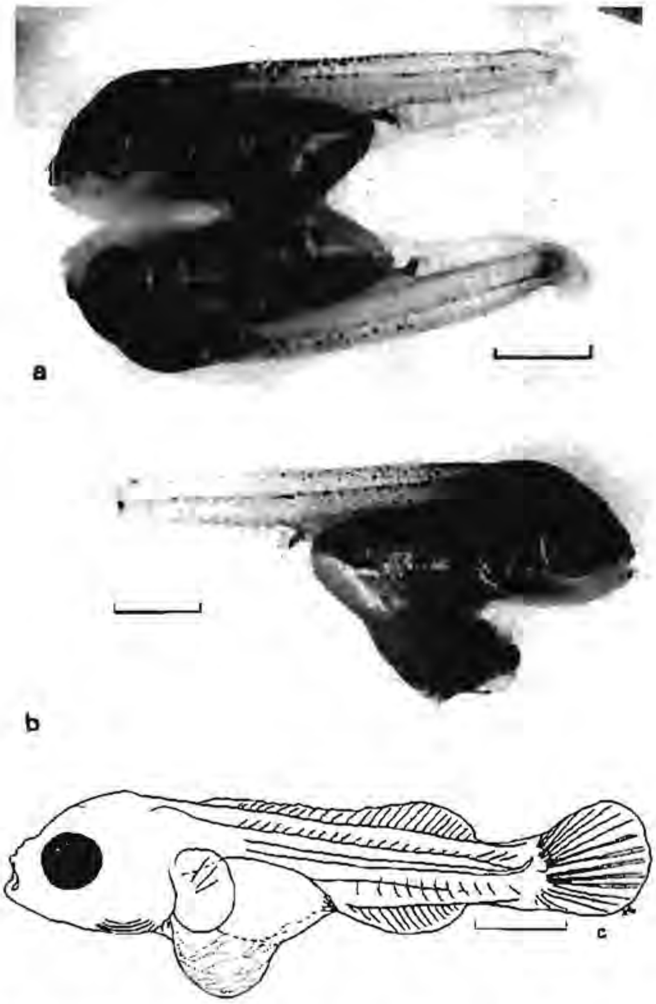

Figure 4 The normal (a) and deformed (b) twins and a normal specimen (c) of *O. mossambicus* at age 8 d 17 h. Scales = 1,0 mm.

Age 7 d 20 h–11 d 15 h

Normal twins: Owing to yolk absorption the lower jaws and the margins of the pericardiums of these individuals were in contact with each other. Little of the yolksac remained (Figure 4a). The gastro-intestinal tract was strongly convoluted and there may have been food in the gut. Development of caudal fin rays and radial loops was similar to that described for the normal specimens and the deformed twins 24 h earlier. Blood pools were seen in the head posterior to the brain and anterior to the eyes of the twin with the deformed tail.

At age 10 d 13 h the full complement of dorsal and anal fin rays was present. Defecation was observed, thus indicating that exogenous feeding had begun.

At age 11 d 15 h the twins had separated, either of their own accord or as a result of removal from the incubator. The pericardium had been ripped and there was a hole in the body cavity. Torn tissue, a remnant of the point of contact, hung below one individual. The level of development was still about a day less advanced than that of the deformed twins and the normal specimens. Death occurred shortly thereafter.

Deformed twins and normal specimens: The level of development of Twin B was almost equal to that of the normal specimens by age 8 d 17 h. Definite dorsal and anal fin rays were present along the remaining finfolds in the normal specimens but were less extensive in Twin B. The snout of Twin A was in contact with the lower branchiostegal rays of Twin B. Twin A had distorted pectoral fins and there was a deceleration of the pericardial pulses (see Figures 4b & c). There were blood streaks along the top and below the notochord but no circulation was detected in the body. Twin B was smaller than the normal specimens but larger than the normal twins.

By age 9 d 16 h Twin A was opaque and its tissue was milky in colour. Branchiostegal rays and pectoral fin rays were present but deformed. Circulation was noted around the gill area and in the head. The blood streaks were thicker and more profuse and blood pools had formed in the head. One normal specimen was slightly more advanced in its development in that circulation was noted in the anal fin and

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Figure 5 The deformed twins at time of death. Although the exact age of the fish is unknown, it is well over 51 d old. Scale = 10,0 mm.

a pelvic fin bud was present.

By age 11 d 15 h the tail of Twin A appeared to be integrated into the body. The finfold was almost completely differentiated and the yolksac nearly totally absorbed in Twin B. Twin A lay butted against the head of Twin B. There had not been any movement noted from Twin A for some time. The swimming ability of the twins seemed to be unhampered and efficient.

Age 11 d 15 h —

At this age the deformed twins were removed from the incubator. They were kept in a 100-l aquarium and checked sporadically. At age 13 d 14 h Twin B had nine, four and five segments in the caudal, anal and dorsal fin rays respectively. The lenses over the eyes of Twin A turned an orangy, opaque colour and the body was milky white with little pigmentation. Twin B was eating and had the appearance of a normal juvenile *O. mossambicus* except for the presence of Twin A which resembled a large growth on its side.

Until 51 d Twin B continued to grow and develop normally. Later the twins were placed in an aquarium with some larger fish which picked at the deformed twin. Shortly afterwards both twins died. The total length of Twin B at this time was 100 mm (Figure 5).

Other abnormalities

Some of the other abnormalities which were noted in specimens from this clutch were: (i) spinal disorders such as distorted caudal peduncles often with endemia, (ii) blood pools within cavernous areas on the yolksac and the consistency of the yolk spongy, (iii) head deformities, often with no distinguishable eyes or other head structures, (iv) enlarged pericardiums often with thin tube-like hearts. A combination of these deformities often existed. A sample of 19 individuals at age 3 d 16 h contained seven normal and 13 deformed specimens. Figure 3c illustrates an embryo at age 5 d 18 h which exhibited several deformities.

Heartrate

In most cases, three readings were taken at a given time to determine mean heartrate of an individual. Table 1 illustrates the mean heartrate of the normal and deformed twins as well as that of normal specimens from the clutch from ages 2 d 19 h to 8 d 17 h. Twin A of the deformed twin showed very irregular and erratic pulses in the pericardial cavity but a distinctive heart was never seen. This made readings difficult and arbitrary in most instances. Heartrates of the normal twins were similar overall. Except for the readings at age 3 d and 8 d 17 h, the heartrates of the normal twins and Twin B were also similar. Heartrates of the normal specimens showed little differences compared to both sets of twins.

The mean heartrate of five individuals which had other abnormalities at age 3 d ranged from 129 to 152 beats/min. At age 5 d 18 h another abnormal specimen had a mean heartrate of 101 beats/min. The former readings were intermediate between both sets of twins, whereas the latter readings were considerably lower.

Discussion

A marked decline in heartrate occurred around the time of first exogenous feeding (Table 1). The same pattern in heartrate was observed in normally developed *O. mossambicus* (Figure 10 in Holden & Bruton 1992) whereby a peak in heartrate occurred followed by a rapid decline once first exogenous feeding had taken place.

Although the pattern of development of the specimens in this study was similar to that of *O. mossambicus* as described by Holden & Bruton (1992), the developmental rate was greater than that of the embryos in their study. Factors which influence development are nutritional content of the eggs, condition of the parent, natural variation of growth rates, genetic differences or incubating temperatures.

Further research is required to clarify the degree of influence these factors may have on developmental rates. The faster developmental rate and growth of the deformed twins as compared to the normal twins may be attributed to the utilization of the available nutrition in the yolk. Development of anatomical structures for the two individuals of the normal twins placed greater demands on the endogenous source of energy, the yolk, and resulted in a slower developmental rate.

Abnormalities in fish embryos can be the result of faulty genetic combinations or disturbances during early development (Reichenbach-Klinke & Elkan 1972). In trout hatcheries, twinning freqently occurs (Behnke & Kloppel 1975). This could be attributed to overripened eggs or unfavourable conditions for development (Sakai, Nomura, Takashima & Oro 1975; Witschi 1952). Dissolved oxygen levels could be a factor in causing twinning. Stockard (in Stephens 1973) caused twinning in *Fundulus heteroclitus* blastoderms by culturing them in oxygen deficient water. Stephens (1973) suggested that the twinning he observed in *Porichthys notatus* could have resulted from the exposure of late cleavage blastoderms to low oxygen levels in nesting areas owing to crowding and/or exposure at low tide. A possible explanation for unequal twinning of a wild caught specimen of *Oncorhynchus mykiss* was attributed to the substrate conditions of nesting sites (Behnke & Kloppel 1975). The compaction of clay particles in some of the nests (redds) could result in reduced water circulation and oxygen levels

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which could have led to abnormal development and twinning. Kanayev (in Moiseyeva 1989) produced *Fundulus* sp. and trout twins by experimenting with low temperatures and ultraviolet light at early embryonic stages. Svyatogor (1989) presents a summary of various conditions and circumstances which lead to polyembryony in fishes and Laale (1984) provides a review and bibliography of this phenomenon. Svyatogor (1989) concludes that the occurrence of polyembryony in fishes is relatively rare but more common under artificial conditions. He considers that this is a response of the early embryonic stages to effects of non-specific trauma.

Exact information about the parental origin of this clutch is not available but it is known that the parent stock came from one of two locations in the Eastern Cape region and had been held in captivity for less than one year. Inbreeding in captivity as a possible cause for these abnormalities can therefore be ruled out. Inbreeding in the wild populations of the parent stock would be unlikely. Two more likely explanations would be traumatization of the eggs when removed from the female's buccal cavity or deficient oxygen levels in the incubator funnels.

To prevent a high incidence of abnormalities in cultured fish, aquarists and aquaculturists should have some knowledge of the requirements of the early embryonic stages of the species they are working with, particularly with regard to oxygen and temperature. Handling of the very early stages should be done cautiously or avoided to prevent any injury or trauma which would increase the probability of abnormalities occurring. The conditions of the breeding and holding facilities should be kept at an optimum. By introducing new or wild individuals to the brood stock and increasing the gene pool, the problems of inbreeding would be minimized.

Although polyembryony occurs more frequently under artificial conditions, it has been reported in wild populations (Von Bonde & Marchand 1929; Stephens 1973; Behnke & Kloppel 1975) but the fish rarely survive to adulthood (Laale 1984). Two recorded exceptions were an unequal twin *Oncorhynchus mykiss* (493 mm total length, 1,7 kg) caught in Lake McConaughy, Nebraska (Behnke & Kloppel 1975) and a double-headed *Salmo trutta* (203 mm) over seven years old (Laale 1984). The deformed twin in this study was also a result of unequal twinning and lived to the juvenile stage. Survival beyond the fry stage is rare but its occurrence could be indicative of beneficial conditions for survival of fish from the juvenile to the adult stage under farm conditions (Bruno 1990). Death of the unequal twin in this study was due to predation. Whether it could have survived under natural conditions is questionable. Usually polyembryonic fish in the wild succumb to abnormalities, injuries or predation (Laale 1984).

Acknowledgements

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References

BEHNKE, R.J. & KLOPPEL, T.M. 1975. An expression of unequal twinning in an adult rainbow trout. *Copeia* 1975 (4): 775–777.

BRUNO, D.W. 1990. Occurrence of a conjoined twin among farmed Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* 37: 501–502.

HOLDEN, K.K. & BRUTON, M.N. 1992. A life-history approach to the early ontogeny of *Oreochromis mossambicus* (Pisces, Cichlidae). *S. Afr. J. Zool.* 27: 173–191.

HUANG, C.M., CHENG, H.J., CHANG, S.L., CHAO, N.H. & LIAO, I.C. 1987. Siamese twins in tilapia. *J. Fish Biol.* 31: 441–442.

HULATA, G. & ROTHBARD, S. 1978. 'Siamese-twins' in *Sarotherodon mossambicus*. *J. Fish Biol.* 13: 45.

LAALE, H.W. 1984. Polyembryony in teleostean fishes: double monstrosities and triplets. *J. Fish Biol.* 24: 711–719.

MOISEYEVA, E.B. 1989. A case of cojoined twins with anterior bifurcation in the Black Sea goby, *Neogobius melanostomus*. *J. Ichthol.* 29: 129–131.

REICHENBACH-KLINKE, H. 1972. Fish pathology. TFH, Hong Kong. 502 pp.

REICHENBACH-KLINKE, H. & ELKAN, E. 1972. The principle diseases of lower vertebrates. Book 1: Diseases of fishes. TFH, Hong Kong. 205 pp.

SAKAI, K., NOMURA, M., TAKASHIMA, F., & ORO, H. 1975. The over-ripening phenomenon of rainbow trout. II. Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. *Bull. Jap. Soc. Sci. Fish.* 41 (8): 855–860.

STEPHENS, R.R. 1973. Twinning in *Porichthys notatus* Girard. *Trans. Amer. Fish. Soc.* 102 (4): 846.

SVYATOGOR, G.P. 1989. Polyembryony in fishes. *J. Ichthyol.* 29 (3): 152–159.

VON BONDE, C. & MARCHAND, J. 1929. Case of 'Siamese twins' in spiny dogfish (*Squalus fernandinus*). *Nature, Lond.* 123: 795–796.

WITSCHI, E. 1952. Overripeness of the egg as a cause of twinning and teratogenesis: a review. *Cancer Research* 12 (11): 763–786.

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