Preliminary Study on Hatching of Rotifers *(Brachionus plicatilis)* and Copepods (Cyclopoida): Response to Flooding and Organic Fertilisation

Margareth S. Kyewalyanga[1], Augustine W. Mwandya[2] and Arie Valdenberg[3]
[1]*Institute of Marine Sciences, University of Dar es Salaam, P. O. Box 668, Zanzibar, Tanzania;*
[2]*Tanzania Fisheries Research Institute, P. O. Box 78850, Dar es Salaam, Tanzania;*
[3]*Maagan Michael Fish Breeding Centre, 37805 Israel*

Key words: rotifers, copepods, mesocosm, hatching, flooding and fertilisation

Abstract— The effect of water flooding, organic fertiliser application and salinity manipulation in stimulating the hatching of rotifer resting eggs was investigated during the dry season (in August/September 1999) in simulation tanks and earthen ponds at Makoba, Zanzibar, . The majority of hatched zooplankton included rotifers, identified as *Brachionus plicatilis,* and other zooplankton such as copepods and protozoa were present in small numbers. The number of rotifers hatched in simulation treatments with low salinity was higher than the values in treatments containing undiluted seawater. Up to 22 ± 1.5 (mean ± SE) rotifers/ml were counted in treatments with low salinity, whereas only a maximum of 13 ± 1. rotifers/ml were found in high-salinity treatments. On the other hand, although salinity in earthen ponds was similar (32–33‰), the ponds flooded and fertilised with chicken manure yielded significantly more rotifers (26 ± 1 / ml), compared to a maximum of only 8 ± 0.5 rotifers/ml counted in the control unfertilised pond. These results suggest that it is possible to induce hatching and production of rotifers by manipulating salinity and fertility of ponds. The reared rotifers can be harvested and used to feed finfish larvae elsewhere.

INTRODUCTION

Several aquatic invertebrates possess the ability to undergo dormancy by producing resting stages as a response to adverse environmental conditions. This dormancy ensures the survival of the species, and is broken when conditions change to favour continued development.

The influence of environmental factors, particularly temperature, salinity, oxygen levels and nutrient concentration on hatching of resting eggs has been studied in zooplankton such as *Artemia* (Sorgeloos, 1980), copepods (Grice & Marcus, 1981) and rotifers (Porriot et al., 1980; Lubzens, 1981). Factors such as oxygen concentration, pH, salinity and nitrite concentration have also been shown to influence the survival and production of rotifers (Schlüter & Groeneweg, 1981). The dormant stage in rotifers is the egg stage (Wallace & Snell, 1991), while in the Cyclopoida (the order to which copepods belong), it is the last copepodite (Williamson, 1991).

Zooplankton forms a major source of live, natural food for several organisms reared in marine aquaculture, including finfish larvae and various invertebrates. The adequate supply of rotifers at the appropriate time is of paramount importance to the survival of many species of fish larvae (Lubzens, 1981). Apart from their high nutritional value, rotifers are relatively small, ranging in size from 0.06 to 1.00 mm, depending on the zoogeographical strain and stage of development (Yufera, 1982; Snell & Corrillo, 1984), making them suitable as first larval feed.

Corresponding Author: MSK.
E-mail: maggie@ims.udsm.ac.tz

In production of live food for feeding fry or fingerlings, there are two main conventional concepts applied, and a modified or unconventional third concept. The first concept is usually known as a 'mesocosm' setup. This includes a variety of procedures and facilities in which the farmer encourages a natural process of zooplankton succession in a particular water body (such as earthen ponds) through either fertilisation or manipulation of environmental factors like salinity and oxygen concentration. The second approach is based on facilities that are usually cut-off from the natural environment and mostly used for marine fingerlings production. The necessary components for the fry feeding are treated separately as a monoculture or as a prepared product of resting eggs collected in nature and hatched when needed. This is mostly done for *Artemia* eggs, but resting eggs of rotifers can also be produced under laboratory conditions (e.g., Minkoff et al., 1983; Hagiwara et al., 1995). The third approach of fry rearing, which is relatively uncommon, includes a combination of the above two concepts.

The present study examined the external conditions necessary for applying the first or the third approaches at the aquaculture site at Makoba, Zanzibar. It explored the levels of organic nutrients and salinity required to stimulate the hatching of rotifers and other zooplankton groups in earthen ponds that could be colonised or used as zooplankton production sites.

Two kinds of complementary experiments were performed, with the following objectives: (i) to determine the response of zooplankton population to water flooding and organic fertilisation—conducted in earthen ponds; and (ii) to assess the effect of salinity manipulation on hatching of zooplankton—conducted in plastic simulation containers.

MATERIALS AND METHODS

Experiments were carried out during late August to early September 1999 at Makoba, a mangrove area located 20 km from Zanzibar town, in the north-west of Unguja Island off the Tanzania coast. The study site was originally used for saltpans. The whole area (ca. 15 ha) is separated from the mangroves by earthen walls. It is confined to a zone limited between high level of neap tide and high level of spring tide. Main components include several small ponds and an operational reservoir that covers about one-third of the total area (For a schematic of the Makoba salt ponds, see Kyewalyanga et al., 2003). Only some of the ponds are protected from tidal flooding, as there are some breaches in the surrounding wall.

The experiments were conducted from 27 August (Day 0) to 1 September 1999 (Day 5). Two kinds of experiments were performed: one examined the effect of water salinity on hatching of zooplankton, specifically rotifers. The other looked at the contribution of increased nutrients (fertilisation) on hatchability or development of rotifers and other zooplankton. Fertilisation with chicken manure stimulated the heterotrophic detrital chain and/or the autotrophic chain by supporting the growth of phytoplankton and algal mats. The fertilisation experiments were conducted in earthen ponds formerly used as saltpans, whereas the salinity treatments were carried out in plastic simulation tanks.

Flooding and fertilisation experiment in earthen ponds

This experiment was conducted in salt ponds, to examine the contribution of flooding and fertilisation (with chicken manure) in inducing hatching or development of zooplankton, mainly rotifers. The idea was to follow the natural succession of zooplankton and monitor changes in abiotic variables (temperature, oxygen concentration, oxygen saturation, nutrient level, etc.), which would then allow an evaluation of the feasibility of producing fry/fingerlings under a mesocosm setup. The treatments were as shown in Table 1.

Each pond was 15 x 12 m, and shallow—ca. 0.25 m deep. The bottom was covered by cyanobacteria mats, which could be seen through the clear water. The ponds were flooded with reservoir water to start the development of zooplankton. Sampling for zooplankton and dissolved inorganic nutrient analysis was done daily, except on Day 2. In all cases, counting was done in triplicate using a custom-made counting chamber, under a dissecting microscope. Sporadic

Table 1. Three ponds set-up to assess the effectiveness of fertilising salt ponds, with chicken manure, for inducing hatching of rotifers and other zooplankton at Makoba

Pond	Treatment	Salinity (‰)
A	Flooded with reservoir water on Day 0+. No addition of manure for the first four days, then 7 litres of manure added on Day 4	~35
B	Flooded with reservoir water and 3 litres of manure added on Day 0. One litre of manure added on Day 1, then 7 litres on Day 4	~35
C	Flooded with reservoir water and 6 litres of manure added on Day 0. Then 2 litres of manure added on Day 1	~35

+'Day 0' is the the day the experiment commenced

samples of adjacent unflooded and unfertilised ponds were taken daily.

Abiotic variables in salt ponds and simulations were also measured. Ammonia and nitrite levels were determined using a simple nutrient kit (MERCK); oxygen concentration, oxygen saturation and temperature were measured using an oxygen meter (Oxy-Guard), and salinity was estimated using a hand refractometer.

Simulation experiments in plastic containers (tanks)

For this experiment, five treatments were run, each with duplicate containers. Five litres of sediments collected from the upper 3–4 cm surface of salt pond fringes were placed in each experimental tank, with a volume capacity of 30 litres (base diameter 35cm, surface diameter 45cm, height 25cm). The containers were filled with seawater from different sources, after filtering through a 45 µm mesh-size net to remove any existing zooplankton. The containers were then immersed into the earthen ponds, such that the pond water level reached up to about 90% of the height of the container. Table 2 summarises the contents of the five treatments. The first three treatments had the same salinity. The aim was to assess if some chemical components that might be present in the fishpond and/or salt pond could induce the hatching process. The last two were diluted with underground fresh water. Dilution was done to test the effect of salinity on hatchability of resting eggs.

Table 2. Five treatments set up to test the effectiveness of the water source in inducing hatching of rotifers and other zooplankton at Makoba

Treatment	Source of water	Salinity (‰)
FISH-35	Water from a grow-out fish pond	35
SALT-35	Water from the salt pond (no fish)	35
RES-35	Water from the reservoir (control)	35
RES-30	Reservoir water, slightly diluted	30
RES-25	Reservoir water, much diluted	25

Sampling from these treatments was also made daily. This involved taking seawater samples for counting rotifers and copepods. Water samples were also taken for dissolved inorganic nutrient (ammonia and nitrite) analysis. On the fourth day of the experiment, all the treatments were fertilised with chicken manure (about 15 ml in each tank) to examine if addition of fertiliser would stimulate hatching. Furthermore, when the population appeared to start decreasing after reaching the peak, 25 and 30% of the water volume in the culture media with zooplankton population was harvested from treatments RES-30 and RES-25, respectively, to assess if this would stop the decline of the zooplankton biomass.

Statistical analysis

The statistical program Statistica® was used to do a correlation analysis to assess the relationship, if any, between the biotic variables as well as change in variables with time, i.e., increase or decrease in the numbers per ml from the start to the end of the experiment. Furthermore, ANOVA and t-test were performed to examine the influence of water sources and change in salinity on hatchability of rotifers in the simulation containers.

RESULTS

The dominant zooplankton species found during the survey were the rotifer *Brachionus plicatilis* and an unidentified copepod from the order Cyclopoida. Rotifers were 150 ± 10µm long, 125

± 5µm wide and the adult copepod was 600 ± 40µm long and 200 ± 10µm wide. Two, relatively small, species of ciliates made up the protozoal population and the most pronounced was a large tintinnid. A few nematodes and Cyanobacteria, *Oscillatoria* sp., were also found. Mud-skippers (*Periophthalmus* spp.) were very common in all saltpans.

Pond experiments

The effect of fertilisation on hatching of resting eggs in the earthen ponds is shown in Fig. 1. As seen, hatching started on Day 1— the day after flooding and application of chicken manure was done. This was indicated by a significant increase in the number of rotifers/ml from Day 0 to Day 1 in fertilised ponds (B and C). For Pond A (unfertilised), only a small increase was observed. However, there was some response due to flooding, with a higher number of rotifers being recorded in flooded ponds than in the sporadic samples collected from unflooded and unfertilised ponds. Ponds B and C, which were both flooded and fertilised on Day 0, yielded more rotifers reaching a maximum of 26 ± 1 and 23 ± 0.2 /ml, respectively.

The increase in rotifers/ml in pond A was slower compared with that recorded in Ponds B and C at a particular time of sampling. For pond C, the rotifer density kept increasing, though more slowly, until the experiment was terminated. The observed increase in rotifer density in Pond A, though small, is indicative of the stimulation of hatching by flooding.

Simulation experiments

In the simulation tanks, hatching of resting eggs and thus increases in the number of rotifers/ml was also observed on Day 1. In all treatments the maximum numbers of rotifers were recorded on Day 4 of the experiments (Table 3, Fig. 2). The addition of 15 ml of chicken manure on Day 4 did not stimulate further growth or maintain the size of the rotifer population; instead, it suppressed it, as illustrated in Fig. 2. Furthermore, harvesting of 25 – 30% of the zooplankton population did not reduce the decline in the population significantly. No significant difference in number of rotifers/ml was found between waters from the fishpond, reservoir and saltpans (ANOVA; $F = 0.084$, $df = 2$, $p>0.05$) and the number of rotifers/ml was also not significantly different between salinity of 25‰ and that of 30‰ ($t = 0.395$; $df = 14$, $p > 0.89$; Table 4).

The highest level of hatching occurred in treatments with lower salinity (25–30‰). with the

Table 3. Average number of rotifers/ml (from two replicates) in the five simulation-treatments for each sampling day. n.d. means no data collected.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
FISH-35	2.5	n.d	4.5	13.0	8.0
SALT-35	4.0	n.d	5.5	13.0	4.0
RES-35	0.5	n.d	3.0	11.0	9.5
RES-30	2.5	n.d	15.0	18.5	9.0
RES-25	3.0	n.d	14.0	23.5	12.0

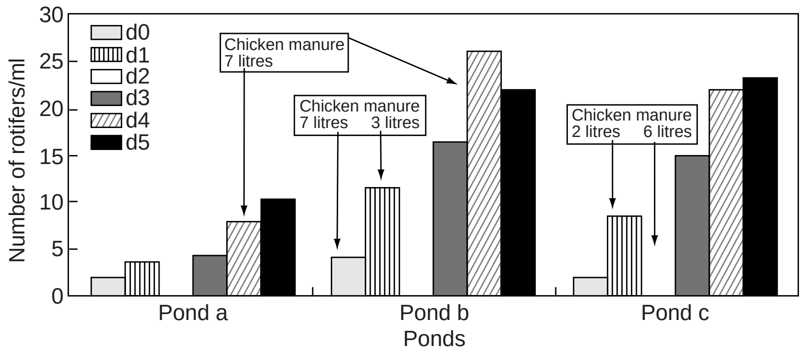

Fig. 1. Number of rotifers/ml in the experimental earthen ponds A, B & C for the six sampling days. d0 is Day 0, d1 is Day 1 etc. Note that there are no data for Day 2, appearing as a gap between the bars. The arrows indicate the day of manure addition, the amounts are also given in the boxes.

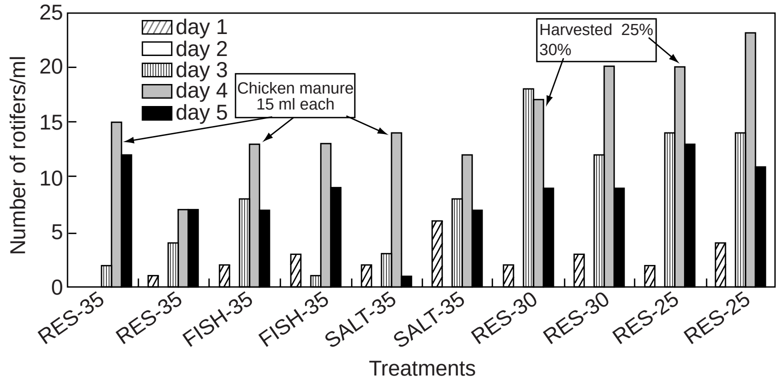

Fig. 2. Rotifer dynamics in simulation tanks for the five sampling days. Symbols (X-axis) are as explained in the text. The arrows show the addition of 15 ml of chicken manure, on Day 4 as well as harvesting of 25-30% of population. The values from replicates are both shown here. Note the peaking of the population on Day 4, and the corresponding decline on the following day.

number of rotifers reaching a maximum of 23 ± 1.5/ml (Fig. 2). The number of rotifers in all treatments decreased significantly after Day 4. Comparing the two treatments with low salinities (RES-30 and RES-25), it was also observed that the number of rotifers/ml was slightly higher at a salinity of 25‰ (in RES-25) than 30‰ (RES-30) (Table 3). This further shows that lower salinity is better for rotifer hatching.

In pond A the total number of copepods did not change much (Fig. 3a). In all ponds, the number of nauplii increased from Day 4 to Day 5 (Fig. 3a–c). The ratios of nauplii: Copepoda + copepodite (cop+cpt) calculated from all samples were 2.39 : 1 and 7.22 : 1. The ratios of rotifers/copepods in the first day of the experiment and towards the end of the experiment (Day 5) were 2.53/l and 1.72/l respectively.

Correlation analyses

The results of the correlation analyses are summarised in Table 5 a–d. In all the ponds, there was a significant positive correlation between the number of rotifers per ml and time from start of the experiment; and a negative correlation between the number of nauplii copepods with time (Table 5a). Rotifers were positively correlated to the total number of copepods and with copepodite stage in fertilised ponds (Table 5b). The total no. of copepods and copepodites were positively correlated (Table 5c), but nauplii copepods and copepodites were negatively correlated (Table 5d).

Table 4. Results from a t-test, on average number of rotifers/ml in simulation treatment with salinity of 25 or 30‰

Salinity (‰)	Rotifers /ml	SD	*t*	*df*	*p*
25	12.6	7.1	0.395	14	0.89
30	11.1	6.7			

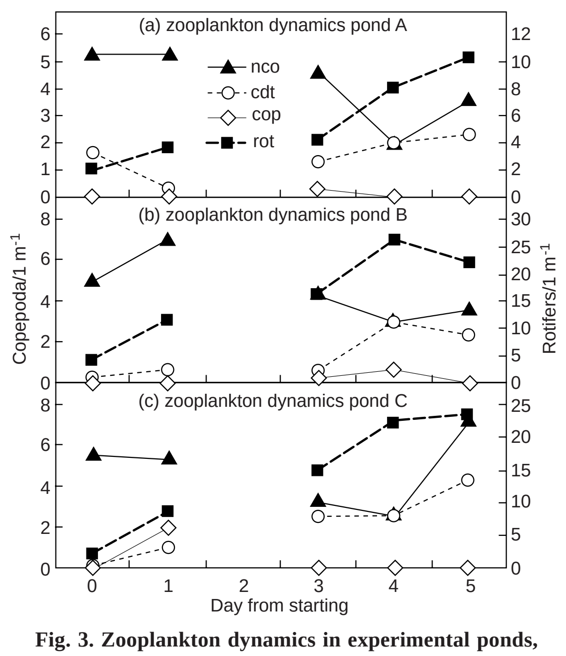

Fig. 3. Zooplankton dynamics in experimental ponds, for the entire sampling period. The top panel shows Pond A, the middle one, showing Pond B and the bottom panel shows Pond C. nco = nauplii copepods; ctd = copepodites; cop = Copepoda and rot = rotifers.

Table 5. Correlation analysis (r-values) in earthen ponds A, B, C and combined (ABC) between (a) Days from start of experiment (D.F.S) and number of organisms per ml; (b) Rotifers (ROT) and the other groups; (c) Total copepods (TCO) and different stages of copepods [copepoda (COP) and copepodites (CDT)]; and (d) Nauplii copepods (NCO) and the remaining two groups. Correlation coefficients that are significant (P < 0.05), are shown in bold typeface.

a) Days from starting				
D.F.S	Pond A	Pond B	Pond C	ABC
ROT	**0.94**	**0.93**	**0.99**	**0.75**
TCO	-0.03	**0.57**	**0.58**	0.38
NCO	**-0.78**	**-0.75**	-0.04	-0.47
CDT	**0.66**	**0.82**	**0.97**	**0.78**
COP	0.11	0.43	-0.43	-0.15
b) Rotifers				
ROT	Pond A	Pond B	Pond C	ABC
TCO	-0.06	**0.78**	**0.54**	0.52
NCO	**-0.78**	**-0.70**	-0.12	-0.25
CDT	**0.69**	**0.90**	**0.93**	**0.73**
COP	-0.22	**0.66**	-0.35	0.01
c) Total copepods				
TCO	Pond A	Pond B	Pond C	ABC
NCO	0.33	-0.32	**0.68**	0.39
CDT	0.46	**0.85**	**0.70**	**0.72**
COP	0.23	**0.53**	0.20	0.33
d) Nauplii copepods				
NCO	Pond A	Pond B	Pond C	ABC
CDT	**-0.67**	**-0.75**	0.14	-0.23
COP	0.18	**-0.62**	0.14	0.05

Abiotic variables

In the earthen ponds, ammonia and nitrite concentrations remained low (between 0 and 0.5 µg-at/l) throughout the experiment. Oxygen concentration and saturation fluctuated over time, ranging from 1.6–8.5 mg/l and 25–135%, respectively. Water temperature ranged from 25.6 to 28.3 ºC. The values for the abiotic variables and dissolved inorganic nutrient concentrations in the simulation experiments were similar to those in the ponds. Oxygen concentration was 1.7– 8.7 mg/l, oxygen saturation 18–136% and temperature 25.1–29.1 ºC.

DISCUSSION

Flooding

Water flooding is essential for hatching of resting eggs, a physiological process that depends on water absorption (Lubzens, 1981). However, there probably must be some 'predictable' clue that triggers the process itself. In all cases, flooding can act as a direct signal (trigger immediately after flooding) or indirect one (some trigger created as a result of processes initiated through interaction between water and exposed sediment). Such a process can be demonstrated by initiation of the detrital food chain caused by interaction of water and organic material trapped in the dry sediments (Harrell and Bokowski, 1990).

The hatching of resting eggs in simulation trials appeared to be a synchronised phenomenon in which most resting eggs hatched in one day to create a dense population in the culture media. A few copepodites and nauplii were found in all tanks of the simulation experiment, so it is not unreasonable to say that resting stages recovered and even completed maturation, eggs extrusion and eggs hatching – all within five days.

The maximum density in all undiluted simulation treatments—reservoir, fishponds and saltpan filling water—was very similar (11, 13 and 13 rotifers/ml, respectively) and indicates similar carrying capacity of rotifers. Similarly, statistical analysis showed no significant difference in the number of rotifers/ml. However, there was more variation in rotifer density on the first day (0.5, 2.5 and 4.0 rotifers/ml, respectively), which could indicate different influences in hatchability of resting eggs. The presence or absence of other organisms in the ponds, for example fish in the fishpond and algal mats in the salt-pans (as opposed to none in the reservoir), may play a role in the hatching of rotifers, although not a significant one.

Fertilisation

The daily three- to fourfold increase in rotifer population (Fig. 1) immediately after fertilisation in ponds B and C respectively, indicates the role of chicken manure fertiliser as a trigger for hatching of resting eggs (see also Schluter &

Groeneweg, 1981). Beyond this effect, chicken manure has an outstanding effect on rotifer population size, at least in the short term. Rotifer density after 5–6 days was more than double in fertilised ponds B and C (> 23/ml) compared to unfertilised pond A (< 11/ml). Doubling the volume of chicken manure added in pond C, as compared with pond B, did not result in better hatching of resting eggs or population size enlargement. Addition of chicken manure on Day 4, the day rotifer population peaked in pond B and in the undiluted tank, resulted in some suppression effect, compared to unfertilised ponds.

In pond A, where the total number of copepods did not change much (Fig. 3a), the correlation between copepodites and number of days was positive, and that between nauplii and sampling day was negative (Table 4a). It was observed that the main response of copepods was by transformation from nauplii to copepodite. In the other two ponds (B and C) the total copepod numbers increased and the correlation between total copepods and copepodites was relatively high (Table 4c). These results point, to some degree, to the recovery of copepodite diapause stages in response to flooding and fertilisation. The increase in nauplii from Day 4 to Day 5, observed in all ponds (Fig. 3a–c) indicated maximum development in 4 days from extrusion.

Production

Harvesting of rotifers on the day of peak population was able to significantly decrease the decline of the population. A 30% harvesting in treatment RES-25 (simulation tanks) was slightly better in preventing population collapse as compared to the 25% harvesting in treatment RES-30 (Fig. 2). It could therefore be assumed that greater manipulation of harvesting would improve these results. In any case the results from manipulation of salt-pans as well as the simulation experiments show good and very rapid response; thus, this means of management of zooplankton is possible.

Abiotic variables

There was no correlation between rotifer density and abiotic variables (nutrients, oxygen concentration and saturation, and water temperature) in the ponds. However, influences of abiotic variables on zooplankton dynamics, such as rotifers and copepods, have been reported in other studies (Ludwig, 2000; Minkoff et al., 1983, Lubzens et al., 1993). The low level of ammonia and nitrate measured suggests that fertilisation with chicken manure, if controlled, does not raise nutrient concentrations to lethal levels. Nevertheless, it is assumed that the sampling duration was too short for a measurable effect to be detected. The high range and fluctuation in oxygen concentration/saturation values suggest that oxygen is one of the important abiotic factors to monitor closely, especially when one has fry or fingerlings in the ponds.

CONCLUSION

This study has demonstrated that 'mesocosm' production of a high biomass of marine zooplankton in earthen ponds at Makoba salt ponds is feasible. Furthermore, the results indicate that the best conditions for obtaining high numbers of zooplankton in Makoba are low salinity, fertilised ponds. We recommend that further experiments be conducted to determine the level and frequency of fertilisation required. Furthermore, available type of phytoplankton and zooplankton as well as their seasonality need to be determined so as to identify the best time/season to start zooplankton nurseries.

Acknowledgements—We are very grateful to the Director, Institute of Marine Sciences (IMS), Zanzibar for providing us with necessary support to conduct the experiments at Makoba. Thanks also go to the Administration at the Maagan Michael Kibbutz, in Israel, for allowing A. Valdenberg to visit IMS. Our sincere gratitude goes to two reviewers, whose constructive comments greatly improved this manuscript. Sida/SAREC Bilateral Programme funded the research.

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