

PERFORMANCE OF ARTEMIA SHELL-FREE EMBRYOS, *Moina micrura* AND PHYTOPLANKTON ON LARVAE OF REARED AFRICAN CATFISH

G.W. NGUPULA, A.P. SHOKO¹, M. MUSIBA, J. NDIKUMANA² and E. ZZIWA²
Tanzania Fisheries Research Institute, P. O. Box 475, Mwanza, Tanzania

¹Tanzania Fisheries Research Institute, Headquarters, P. O. Box 9750, Dar es Salaam, Tanzania

²Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA),
P. O. Box 765, Entebbe, Uganda

Corresponding author: ngupula@yahoo.co.uk

ABSTRACT

Starter feeds are important in the growth of African catfish *Clarias gariepinus* (Burchell) larvae. A study was conducted to investigate the performance of Artemia shell-free embryos, *Moina micrura* (Kurz) and phytoplankton as starter feed for larvae rearing of *C. gariepinus*. The experiment lasted 15 days in a set of nine tanks of 900-litre capacity. The study was divided into two phases of 5 and 10 days. During the end of the 15 days, the larvae fed on *M. micrura* had a growth rate of $32.95 \pm 12.62\%$ day⁻¹, survival of $76.51 \pm 7.33\%$ and Specific Growth Rate (SGR) of 0.17 ± 0.00 . The larvae fed on Artemia exhibited a growth rate, survival and SGR of $85.5 \pm 74.4\%$ day⁻¹, $97.71 \pm 0.00\%$ and 0.28 ± 0.18 , respectively. The larvae fed on phytoplankton exhibited a growth rate, survival and SGR of $36.1 \pm 44.58\%$ day⁻¹, $45.73 \pm 24.14\%$ and 0.12 ± 0.11 , respectively. The difference in performance between the feeds was mainly due to differences in their nutrient composition and levels. This study recommends the use of Artemia embryos as the best starter feed for the rearing of *C. gariepinus* larvae followed by the *M. micrura*.

Key Words: *Clarias gariepinus*, growth rate, specific growth rate, survival

RÉSUMÉ

Les aliments d'entrée sont importants pour la croissance des larves du poisson-chat Africain *Clarias gariepinus* (Burchell). Une étude était menée à la station de recherche de l'Institut de Recherche sur la Pêche (TAFIRI) de Mwanza en Tanzanie pour évaluer la performance des embryons dénudés d'Artémia, *Moina micrura* (Kurz) et les phytoplanctons comme aliments d'entrée pour la croissance des larves de *C. gariepinus*. L'expérience a duré 15 jours dans un ensemble de neuf réservoirs d'une capacité de 900 litres. L'étude était subdivisée en deux phases de 5 et 10 jours. Vers la fin de ces 15 jours, les larves nourries de *M. micrura* avaient un taux de croissance de $32.95 \pm 12.62\%$ par jour, $76.51 \pm 7.33\%$ de survie et un taux de croissance spécifique (SGR) de 0.17 ± 0.00 . Les larves nourries sur base d'Artémia ont montré un taux de croissance ; un taux de survie et une SGR de $85.5 \pm 74.4\%$ par jour, $97.71 \pm 0.00\%$ et 0.28 ± 0.18 , respectivement. Les larves nourries de phytoplanctons ont manifesté un taux de croissance, une survie et un SGR de $36.1 \pm 44.58\%$ par jour, $45.73 \pm 24.14\%$ et 0.12 ± 0.11 , respectivement. La différence en termes de performance entre les aliments utilisés était principalement due aux différences dans leur composition en matière nutritives et concentration minérales. Cette étude recommande l'utilisation d'embryons d'artémia comme meilleur aliment d'entrée pour la croissance des larves de *C. gariepinus*, suivie de *M. micrura*.

Mots Clés: *Clarias gariepinus*, taux de croissance, taux spécifique de croissance, survie

INTRODUCTION

Aquaculture has been practiced since 1940s in East Africa, but the industry remains relatively undeveloped largely due to dependence on aquatic products from capture fisheries. Currently, due to decline in most of the capture fisheries and increased demand for protein of aquatic products, the need for an alternative source, particularly from aquaculture is growing. One of the major obstacles towards development and the ultimate takeoff of the aquaculture industry is lack of technologies and availability of affordable and quality fish seed and feed (FAO, 2006; Mwanja *et al.*, 2006). The need for quality fish seed for aquaculture production and availability has proved a challenge due to the associated larval complications during their ontogenetic stages (Luizi *et al.*, 1999).

Since most larvae of marine and some fresh water species such as *Clarias gariepinus*, (Burchell 1822) do not have a functional digestive system at the start of exogenous feeding (Govonni *et al.*, 1986; Luiz *et al.*, 1999), feeding them with food containing simple compounds such as brine shrimps (*Artemia*), rotifers (i.e., *Brachionus plicatilis* (Müller 1786), cladocerans (i.e., Daphnids, *Moina micrura* (Kurz 1874)) and phytoplankton have been employed with successful outcomes (Govonni *et al.*, 1986; Lubzen *et al.*, 2001; Hamre, 2006; Olurin *et al.*, 2012). Normally, most live feeds used perform differently in different larval types (Lubzen *et al.*, 2001; Hamre, 2006; Olurin *et al.*, 2012). Therefore, it is important that any live feed type is tested first for its performance before it is assigned for a particular species.

Although *Artemia nauplii* and decapsulated cysts have for long been used successfully in first feeding of most fish larvae (Luizi *et al.*, 1999; Yilmaz *et al.*, 2006; Olurin *et al.*, 2012), their increasing cost is a constraint to most of the poor subsistence farmers, especially in sub-Saharan Africa. In this regard, efforts towards the search for alternative feed sources have been ongoing. Although copepods are much more nutritious than rotifers and cladocerans in larval culture, the preference is more for rotifers and cladocerans mainly because of their relative easiness of mass culture (Luizi *et al.*, 1999; Evjemo *et al.*, 2003;

Mckinnon *et al.*, 2003). In addition, the cladoceran zooplankton of the genus *Moina* have for long been used as a starter food for the larvae of most fish like *Chanos chanos* and *Clarias macrocephalus* (Yilmaz *et al.*, 2006).

This study aimed at exploring the performance of *Artemia* shell-free embryos, *M. micrura* and phytoplankton as feed for larvae of African catfish (*C. gariepinus*). It was hypothesized in this study that *C. gariepinus* larvae fed on *Artemia* shell-free embryos will attain higher growth performance than those fed on *M. micrura* and phytoplankton.

MATERIALS AND METHODS

The experiment was carried out at the Tanzania Fisheries Research Institute (TAFIRI) located at Mwanza, Tanzania from 10 March 2013 to 25 March 2013. Three African catfish females and four males were subjected to artificial propagation (Olumuji and Mustapha, 2012) to lead to larvae for the study. During the implementation of this study, it was difficult to get enough sperms from individual males to fertilise the eggs, which necessitated the use of more males than females. Five days old post-hatch larvae (already exhausted their egg yolk and ready for exogenous feeding) were used to test the performance of *Artemia* shell-free embryos, *M. micrura*, and phytoplankton feeds.

Initially, the larvae were randomly picked and distributed equally into nine experimental tanks (white plastics of 900 litre capacity). Each of the nine tanks contained 800 larvae. Before the start of the experiments, 20 larvae were randomly collected from each of the tanks, and their individual weights and total lengths measured using a 110 g capacity and 0.0001g sensitivity balance (MODEL ADVENTURE PRO AV114C, USA) and a 30-cm ruler, respectively. The tanks (each feed treatment replicated thrice) were maintained on flow-through-system using water directly pumped from Lake Victoria, then filtered, aerated, and allowed to flow under gravity. The larvae in the experimental tanks were fed to satiation three times a day, at 09.00, 13.00 and 16.00 hr. Concurrently with feeding, water quality parameters (pH, dissolved oxygen, and temperature) were monitored three times a day.

Temperature and pH were measured using a portable pH-Temperature meter (HI 991300 pH/EC/TDS/Temperature meter, USA), while dissolved oxygen (DO) was measured using a portable Oxygen meter (HI 9143 Microprocessor Oxygen meter HANNA instruments, USA).

The feeds performance experiment lasted 15 days and was divided into two phases. The first phase, which lasted five days involved feeding the larvae in their respective replicate tanks with Artemia, *M. micrura*, and phytoplankton per se. The second phase (the weaning phase) lasted 10 days and involved supplementing the larvae with a formulated diet (Ugachick: 35% crude protein, 7% lipid, 6.5% crude fibre, 7% ash and 11% moisture), an acclimatisation process towards feeding them with formulated feeds. Ugachick supplementary feed was obtained from Ugachick Poultry Breeders Ltd, based in Uganda. At the end of each phase, the larvae in the nine tanks were randomly sampled to obtain 30 larvae, which were weighed using a 110 g capacity and 0.0001g sensitivity balance, MODEL ADVENTURE PRO AV114C. The total lengths of the larvae were also measured to the nearest 0.1 millimetre using a 30-cm ruler. The faeces and uneaten feed at the bottom of each tank, including the dead larvae were syphoned out daily at 08.00 hr, using a 1 cm thick and 5 m long plastic pipe. The dead larvae were counted for calculation of percentage survival, while the live larvae were separated from the impurities and returned to the tanks.

Growth performance indices. Growth parameters were determined using both length and weight following the formulas provided in Olurin *et al.* (2012):

$$GR = 100 \times (W_f - W_i) / (T \times L_i) \dots\dots\dots (i)$$

$$GR = 100 \times (L_f - L_i) / (T \times L_i) \dots\dots\dots (ii)$$

$$SGR = (\ln W_f - \ln W_i) / (T_2 - T_1) \dots\dots\dots (iii)$$

$$SGR \text{ (mm/day)} = (\ln L_f - \ln L_i) / (T_2 - T_1) \dots\dots\dots (iv)$$

$$SR (\%) = 100 \times N_s / N_i \dots\dots\dots (v)$$

Where:

- GR = growth rate,
- SGR= specific growth rate,
- SR = survival rate,
- W_f = final weight (mg),
- W_i = initial weight (mg),
- L_f = final length (mm),
- L_i = initial length (mm), and
- T = time in days

T₁ and T₂ represent initial and final time (days); N_s and N_i represent number of survivors and initial number of fish, respectively.

The condition factor of *C. gariepinus* larvae was calculated according to Madu *et al.* (2003):

$$CF = 100 \times W/L^3 \dots\dots\dots (vi)$$

Where: w = weight of fish in mg, L = length of fish in mm.

Feed preparation and application. The *M. micrura* cultured in the six black plastic tanks (900-litre capacity) were filtered and concentrated using a 60 µm zooplankton net to make a solution with a density of >2000 individuals in a litre of water, prior to being fed to *C. gariepinus* larvae. Nine litres of the Moina solution were prepared for feeding *C. gariepinus* larvae in the Moina treatment tanks in each feeding day. The *C. gariepinus* larvae in each tank were supplied with 1 litre of the solution three times a day at 09.00, 13.00 and 16.00 hr making a total of three litres per day per each tank.

The phytoplankton used as feed in this study was obtained from three 900 litre-capacity black plastic tanks, which were initially applied with 250 g of urea and lake water (containing phytoplankton) and then left for two months for algae to develop. The algae in the tanks peaked and collapsed from time to time before it changed to some naked eye visible particles. Water in the tanks was first homogenised using a 5 cm thick and 1 m long stick and then filtered for debris and some water insects that otherwise would scare or predate on the larvae. Twenty litres of phytoplankton were then added to the three tanks to test its performance as feed for *C. gariepinus* larvae. The feeding protocol (specified time interval) followed above was also adopted here.

The Artemia shell-free embryos (INVE Aquaculture, INVE (Thailand) LTD. www.ive.com) were imported from Durante Fish Company in Nigeria, at an average price of US\$ 45 per tin of 500 g. A tin of 500 g was enough to rear an average of thirty thousand African catfish larvae in 2 weeks. During each feeding interval, Artemia were weighed and then fed to larvae by smearing on the surface of the water in the tanks.

Samples of the dried *M. micrura*, phytoplankton and Artemia shell-free embryos were proximate analysed for crude protein, lipid, crude fibre, and ash contents.

Statistical analysis. A One-way Analysis of Variance (ANOVA) was used to test for significant differences in growth performance, water quality parameters among the treatments, and in nutrient composition among different experimental diets. Post hoc analysis was done using Tukey HSD test (Zar, 1999). The analyses were done using SPSS version 17 (SPSS Inc, USA). The p-value was set at < 0.05.

RESULTS

During the 1st phase of the experiment, there were no significant differences ($P>0.05$) in growth of the three different tested feeds (Table 1). However, *C. gariepinus* larvae fed with Artemia shell-free embryos had the highest growth rate, while the larvae fed with phytoplankton tended to have the poorest respective parameters. During this phase, pH values ranged from 7.36 ± 0.34 to 7.85 ± 0.014 , while DO and temperature

ranged from 4.17 to 5.86 mg O₂ L⁻¹ and 27.30 to 28.50 °C, respectively. There was no significant difference between the measured water quality parameters ($P>0.05$).

During the 2nd phase (weaning) of the experiment, there were significant differences in growth performances of the African catfish larvae ($P = 0.007$, Table 2), with larvae fed on phytoplankton performing best (Table 2). The catfish larvae fed with phytoplankton had the highest growth rate (67.62% day⁻¹) and SGR (0.2), however, with lowest survival percentage (28.66%). During this phase pH values indicated a range of 6.66 ± 0.12 to 6.89 ± 0.04 , while DO and temperature ranged from 3.84 ± 0.08 to 4.89 ± 0.01 mg O₂ L⁻¹ and 23.15 ± 0.07 to 25.90 ± 0.14 °C, respectively. There was no significant difference between the measured water quality parameters.

On average, Artemia-shell free embryos performed the best, *Moina micrura* the second and phytoplankton the last (Table 3). There was significance in differences of all the growth parameters, except for conditional factor ($P=0.005$).

Artemia had the highest crude protein content (41.89%), followed by *M. micrura* (38.25%) and phytoplankton (14.36%) (Table 4, $P<0.01$).

DISCUSSION

Artemia shell-free embryos performed best in the larvae rearing of *C. gariepinus*, which can be attributed to the fact that this feed had the best nutrient composition (i.e. highest protein and lowest ash content) compared with *M. micrura*

TABLE 1. Growth rate, SGRs, and percentage survival of the *Clarias gariepinus* larvae fed with Artemia shell-free embryos, live *Moina micrura*, and phytoplankton during the 1st phase (the first five days) in a feeds experiment in Tanzania

Parameter	Artemia	<i>Moina micrura</i>	Phytoplankton
Initial average weight (mg)	3.28±0.01	3.28±0.01	3.28±0.01
Final average weight (mg)	25.93±5.02	7.22±1.17	4.03±0.57
Initial average length (mm)	7.00±0.00	7.00±0.00	7.00±0.00
Final average length (mm)	14.2±0.78	11.20±0.84	9.57±0.79
Growth rate (% day ⁻¹)	138.11	24.02	4.57
SGR	0.41	0.16	0.04
Percentage survival	97.70	81.70	62.8
Condition factor (CF)	0.906	0.514	0.459

TABLE 2. Performance of *Clarias gariepinus* larvae fed with Artemia shell-free embryos, live *Moina micrura*, and phytoplankton during the 2nd phase (the last 10 days or the weaning phase) in a feeds experiment in Tanzania

Parameter	Artemia	<i>Moina micrura</i>	Phytoplankton
Initial weight (mg)	25.93±5.02	7.22±1.17	4.03±0.57
Final weight (mg)	111.23±41.10	37.46±8.13	31.28±10.78
Initial length (mm)	14.2±0.78	11.20±0.84	8.23±0.76
Final length (mm)	20.60±1.58	13.60±2.27	13.20±2.28
Growth rate (%/day) (nsd)	32.89	41.88	67.62
SGR(sd)	0.15	0.17	0.2
Survival (%) (sd)	97.71	71.32	28.66
Condition factor (CF) (sd)	1.27	1.49	1.36

nsd = not significant difference, sd = significant difference

TABLE 3. Average growth rate, specific growth rate, and survival percentages of *Clarias gariepinus* larvae fed with Artemia shell-free embryos, live *Moina micrura*, and phytoplankton

	Artemia	<i>Moina micrura</i>	Phytoplankton
Initial average weight(mg)	3.28±0.01	3.28±0.01	3.28±0.01
Final average weight(mg)	111.23±41.10	37.46±8.13	31.28±10.78
Initial average length (mm)	7.00±0.00	7.00±0.00	7.00±0.00
Final average length (mm)	20.60±1.58	13.60±2.27	13.20±2.28
Growth rate (%/day) (sd)	85.5±74.4	32.95±12.62	36.1±44.58
SGR(sd)	0.28±0.18	0.17±0.00	0.12±0.11
Survival (%) (sd)	97.71±0.00	76.51±7.33	45.73±24.14
Condition factor (CF) (nsd)	1.10±0.26	1.00±0.69	0.90±0.64

nsd = not significant difference, sd = significant difference

TABLE 4. Proximate analysis of feeds used in a study of catfish in Tanzania

Feed	Crude protein (%)	Lipids (%)	Crude fibre (%)	Dry matter (%)	Ash (%)
Artemia	41.89	1.55	3.35	89.26	10.89
<i>M. micrura</i>	38.25	6.61	9.51	94.33	44.41
Phytoplankton	14.36	3.74	13.62	87.32	24.14

and phytoplankton. The Artemia shell-free embryos had, as well, some special advantages over other live feeds used. For example, the product was clean and easily administered to fish in specified amounts without requiring further processing; and could safely be stored for longer time periods. The only major constrain with the feed was that it was not available in the East African markets, thus the need for importation.

The finding that Artemia was superior in our study corroborates with that of Olurin and Oluwo (2010) and Olurin *et al.* (2012) who compared the performance of decapsulated Artemia, copepods, and a commercial diet. Their work attributed the best performance of Artemia to the fact that it had balanced nutrient composition compared to other feeds. Generally, Artemia has been appreciated worldwide as a good starter feed for

the larvae of most fresh water and marine fish (Harzevilli *et al.*, 2004; Olurin and Oluwo, 2010; Olurin *et al.*, 2012).

The *M. micrura* feed ranked second after Artemia shell-free embryos. This finding corresponds with the documented nutrient composition of the feeds, which was best for Artemia and *M. micrura* the second. The possibility that *M. micrura* was a bit less palatable compared to Artemia cannot be ignored as *C. gariepinus* larvae when feeding depend heavily on chemosenses rather than visual or mechanical senses (Mukai and Seng Lim, 2011). The possibility that the larvae had small mouths to ingest some big sized *M. micrura* is not a possibility. Yilmaz *et al.* (2006) indicated that African catfish larvae normally have mouths big enough to ingest some big sized zooplankton, such as copepods and cladocerans. Fish larvae of one week old of common carp failed to ingest the *M. micrura*, but ingested higher number of rotifers (Yilmaz *et al.*, 2006). The suitability of zooplankton of the genus *Moina* as a good starter feed for fish larvae was also recommended by Hashim and Ali (1990) and Adeyemo *et al.* (1994).

M. micrura contained 38.25% crude protein, 6.61% crude lipid, 9.51% crude fibre and 44.41% ash (Table 4). Our finding differ from those of Hashim and Ali (1990) that *M. micrura* contained 60.72% crude protein, 18.12% crude lipid, 9.95% carbohydrate, 11.21% ash, 7.81 dry matter and 93.46% moisture. This suggests that the nutrient composition of the cultured *M. micrura* normally vary depending on geographical locations.

The phytoplankton exhibited the poorest growth and survival which can be attributed to the fact that this feed had poorest protein and highest ash content. The fact that phytoplankton does not make a good feed to the larvae of *C. gariepinus* was also observed by Yilmaz *et al.* (2006). Thus, when using phytoplankton as a feed it is important to consider other supplement feeds such as rotifers and *M. micrura*. The lowest survival rate observed in this study could be attributed mainly to cannibalism due to large differences in size, suggesting that the weak and small sized larvae were selectively eliminated by the strong ones (Olurin and Oluwo, 2010; Marimuthu, 2011; Olurin *et al.*, 2012).

ACKNOWLEDGMENT

This publication is a product of a project funded by the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). The views expressed are not necessarily those of ASARECA.

REFERENCES

- Adeyemo, A.A., Oladosu, G.A. and Ayinla, A.O. 1994. Growth and survival of fry of African catfish species, *Clarias gariepinus* Burchell, *Heterobranchus bidorsalis* Geoffrey and *Heteroclarias*, reared on *Moina dubia* in comparison with other first feed sources. *Aquaculture* 49(3-4): 209-221.
- Evjemo, J.O., Reitan, K.I. and Olsen, Y. 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. *Aquaculture* 227 (1-4): 191-210.
- FAO. 2006. Food and Agriculture Organisation. Regional Review on Aquaculture Development in Sub-Saharan Africa. FAO. Fisheries Circular No. 1017/4. Rome, Italy, 93pp.
- Govoni, J.J., Boehlert, G.W. and Watanabe, Y. 1986. The physiology of digestion in fish larvae. *Environmental Biology of Fishes* 16:59-77.
- Hashim, R. and Ali, A. 1990. The use of live, frozen and potassium permanganate. Treated *Moina micrura* for catfish (*Clarias macrocephalus*) larvae. *Pertanika* 13(3): 367-370.
- Harzevili, A.S., Vught, I., Auwerx, J. and De Charleroy, D. 2004. Larval rearing of Ide (*Leuciscus idus* L.) using decapsulated *Artemia*. *Archives of Polish Fisheries* 12:191-195.
- Hamre, K. 2006. Nutrition in cod (*Gadus morhua*) larvae and juveniles. *ICES Journal of Marine Science* 63:267-274.
- Luizi, F.S., Gara, B., Shields, R.J. and Bromage, N.R. 1999. Further description of the development of the digestive organs in Atlantic halibut (*Hippoglossus hippoglossus*) larvae, with notes on differential absorption of copepod and *Artemia* prey. *Aquaculture* 176:101-116.

- Lubzen, E., Zmora, O. and Barr, Y. 2001. Biotechnology and aquaculture of rotifers. *Hydrobiologia* 446/447: 33-353.
- Madu, C.T., Okwuego, C.C. and Madu, I.D. 2003. Optimum Dietary protein level for growth and gonadal maturation of female *Heterobranchus Longifilis* (velenciennes 1840) Brood stock. *Journal of Aquatic Science* 18:29-34.
- Marimuthu, K., Umah, R., Muralikrishnan, S., Xavier, R. and Kathiresan, S. 2011. Effect of different feed application rate on growth, survival and cannibalism of African catfish, *Clarias gariepinus* fingerlings. *Emirate Journal of Food Agriculture* 23 (4):330-337.
- Mukai, Y. and Seng Lim, L. 2011. Larval rearing and feeding behavior of African Catfish, *Clarias gariepinus* under dark conditions. *Journal of Fisheries and Aquatic Sciences* ISSN 1816-4927.
- Mwanja, W.W., Akol. A., Abubaker. L., Mwanja, M., Msuku, B.S. and Bugenyi, F. 2006. Status and impact of rural aquaculture practice on the Lake Victoria basin wetlands. *African Journal of Ecology* 45:165-174.
- McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G. and Robino, B. 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. *Aquaculture* 223:89-106.
- Olurin, K.B. and Oluwo, A.B. 2010. Growth and survival of African catfish (*Clarias gariepinus*) larvae fed decapsulated *Artemia*, live *Daphnia*, or commercial starter diet. *Israel Journal of Aquaculture* 62(1):50-55.
- Olurin, K.B., Iwuchukwu, P.O. and Oladapo, O. 2012. Larval rearing of African catfish, *Clarias gariepinus* fed decapulated *Artemia*, wild copepods or commercial diet. *African Journal of food science and technology* 3(8):182-185.
- Olumuji, O.K. and Mustapha, M.K. 2012. Induced Breeding of African Mud Catfish, *Clarias gariepinus* (Burchell 1822), using Different Doses of Normal Saline Diluted Ovaprim. *Journal of Aquaculture Research and Development* 3(4):1-4.
- Yilmaz, E., Bozkurt, A. and Gokcek, K. 2006. Pre-selection by African catfish *Clarias gariepinus* (Burchell, 1982) larvae fed with different feeding regimes. *Turkey Journal of Zoology* 30:59-66.
- Zar, J.H. 1999. Biostatistical Analysis. 3rd Edition. Northern Illinois University, DeKalb, USA. 663pp.