RESEARCH PAPER



Comparative Study of Reproductive Traits in Gangetic Hairfin Anchovy, *Setipinna phasa* (Hamilton, 1822) from Estuarine and Freshwater Ecosystems

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Abstract

The Gangetic hairfin anchovy, Setipinna phasa (Hamilton, 1822) were collected from river Ganga (Kanpur) and Hooghly estuary (Kolkata) to study the comparative reproductive traits (sex ratio, spawning season, gonado-somatic index, fecundity and egg diameter). Female outnumbered the male in the population of river Ganga but it was non-significant in the population of the estuary. Five maturity stages were identified in males and females of the fish of both ecosystems. Occurrence of multimodal (immature, maturing and mature) and uni-modal (matured) type of ova in the ovary of the fish of Hooghly estuary and river Ganga respectively and the values of gonado-somatic index confirmed the double and single spawning in a year in the fish in estuary and river respectively. The absolute fecundity was found to be higher in the fish of river Ganga compared to that of the estuary (P < 0.05). Linear and positive correlations between fecundity and the total length, body weight, ovary length and ovary weight were noted in the fish of both ecosystems (R² > 0.90). The present study provides the comparative account of the reproductive and spawning strategies of S. phasa for the first time in favour of the proper management and conservation of the fish.

Introduction

The study of the reproductive cycle and fecundity is essential and fundamental for understanding the fish population dynamics to obtain a sustainable harvesting (Murua *et al.*, 2003; Cao *et al.*, 2009). The study of population characteristics and reproduction of the fish is considered to be important for the assessment of commercial potentialities of the fish stock, life history and its management, because management procedures rely entirely upon the reproductive potential of the fish (Doha, 1970; Froese, 2004; Morgan, 2008; Eyo *et al.*, 2014). The study of the reproductive traits includes the knowledge of the size of the fish at first maturity, spawning, sex-ratio, duration of the reproductive season, gonado-somatic index (GSI), ova diameter and fecundity (Murua *et al.*, 2003; De Carvalho *et al.*, 2009; Fontoura *et al.*, 2009). The reproductive study also helps to reveal the differences between the fish stocks of different ecosystems characterized by different environmental conditions (Begg, 1998) and the information so derived are considered to be useful for the artificial breeding of the fish to produce fry and fingerlings on demand (Kamanga *et al.*, 2012). The size at first maturity and fecundity of the fish show an adaptive response to the ecological gradients to ensure the survival of the species to enhance individual fitness (Blanck & Lamouroux, 2007; Guèye *et al.*, 2012).

Setipinna phasa is popularly known as Gangetic hairfin anchovy which belongs to the family Engrulidae and order Clupeiformes. The fish is carnivorous in nature; mainly subsist on the feeds available in column and surface of the water of the habitats. The fish. S. phasa is known to occur in the coasts of Bengal and Orrisa, entering estuaries and rivers through the tidal water. Among the various species of Setipinna, S. phasa has restricted distribution from the Ganges riverine system to the coastal waters of West Bengal (Jones & Menon, 1952; Whitehead, 1972; Whitehead et al., 1988). Setipinna phasa is a pelagic fish which shows amphidromous and potamodromous migration and form one of the major fisheries along the north-east coast of India (Saigal et al., 1987). The species has been classified under 'Least Concern' category (IUCN, 2010). The fish is quite popular as a table fish and nutritious too and hence, considered as commercially important. In spite of the high demand of the fish no any commercial culture has been carried out so far because of the scanty information on the biology of this species. The abundance of the fish is dwindling because of reckless exploitation due to illegal fishing and the anthropogenic pollution of rivers and estuaries. The fish has been the object of very few studies despite its palatability and high consumer demand; Jones and Menon (1952) studied the life history, bionomics and fishery while maturity and fecundity were studied by Jhingran (1961). The morphological variations discernible by multivariate analysis and anomalies in pectoral and pelvic fins have been reported by Gangan *et al.* (2016, 2018a, 2018b). Keeping in mind the paucity of information on the reproductive biology of *S. phasa*, the present study was undertaken on comparative examination of reproductive characteristics of *S. phasa* from two different ecosystems (river and estuary).

Materials and Methods

Study Area

The river Ganga emerges from the Gangotri glacier at Gomukh (30°36' N; 79°04' E) which is about 25.2% of the total freshwater resources of India. The river shows high fluctuations in the rate of water flow within the catchment area and the mean maximum rate of water flow of the river Ganga is 468.7×109 m³ (Sarkar *et al.*, 2012a). The two major distributaries of the river Ganga are Bhagirathi (India) and Padma (Bangladesh). The Bhagirathi flows west and south-west of Kolkata and is known as Hooghly branch and finally drains towards Bay of Bengal. The southern portion of the river is known as Hooghly estuary (21°40'N; 87°47'E) which is the first deltaic derivative of the river Ganga. The Hooghly estuary has a catchment area of 69,104 sq. km and well mixed with seawater due to its shallow depth of less



Figure 1. Map of the sampling sites, S1=river Ganga and S2=Hooghly estuary.

than 6 m (Abbas & Subramanian, 1984; Sadhuram *et al.*, 2005). The distribution map of the sampling sites is given in Figure 1.

Sampling

A total of 1,023 (466 males of size ranged between 70-240 mm and 557 females of size ranged between 70-250 mm) individuals from river Ganga and 1,069 (511 males of size ranged between 60-320 mm and 558 females of size ranged between 60-340 mm) individuals from Hooghly estuary were collected using small meshed (3-4.5 cm in size) cast and drag nets. The monthly sample, comprising 30-60 individuals of *S. phasa,* was procured from both the ecosystems during January-December 2017 and brought to the laboratory packed in ice.

The individuals of the fish were initially cleaned with distilled water and their total length (TL) and eviscerated body weight (BW) were measured using a digital calliper and electronic balance sensitive to the nearest 0.1 mm and 0.1 g respectively. Sex of the fish was determined on the basis of different colouration of the pectoral and caudal fins, reddish swollen genital papilla, soft and bulging abdomen (Winn, 1958a & b; Page, 1974). The weight of the cleaned gonads of the fish was measured using an electronic balance sensitive up to 0.001 g. The ovaries of all the samples were preserved in 4% formaldehyde solution after recording their weight (OW) and length (OL).

Maturity Stages

The maturity stages were ascertained on the basis of the morphological structure of the gonads, space engaged by them in the abdominal cavity and the diameter of unspawned eggs (Nikolsky, 1963; Reddy 1979; Sarkar et al., 2019). Besides, the percentage of occurrence of the ripe individuals was determined during spawning season (Prasad et al., 2011; Sarkar et al., 2019). The gonadal maturation was studied under dissecting microscope, and categorized into one of the five stages of maturity as suggested by Brown-Peterson et al. (2011): I (immature), II (developing), III (spawning capable), IV (regressing) and V (regenerating). Intraovarian eggs were measured at different stages of maturity. The diameter of 40-50 ova per month was measured with the help of ocular micrometre using 8 x12.5 magnifications under a binocular dissecting microscope along the longest axis of ova as suggested by Clark (1934).

Gonado-Somatic Index

GSI of males and females of the fish of each month was computed to determine the gonadal development and frequency of spawning as per Afonso-Dias *et al.* (2005) using the formula:

GSI=100 x OW/BW

Where, OW is the fresh ovary weight and BW is the eviscerated body weight of fish

Fecundity and Condition Factor

The fecundity study was based on the examination of ripe gonads. The total weight of each ovary of all ripe female fish was taken and 100 mg sub-samples of eggs were removed from the anterior, middle and posterior regions of each ovary. The number of ova present in subsamples was counted and the average number of subsamples was multiplied by the OW to calculate the absolute fecundity. Relative fecundities (number of ova in-unit TL, BW, OL and OW) were also recorded. Besides, the relationship of fecundity with various body dimensions, gonadal dimensions was also worked out using linear regression analysis. The condition factor (K) was calculated using the formula as suggested by Nash *et al.* (2006):

$$K = BW*100/TL^{3}$$

Where, BW = is the eviscerated body weight of fish in g and TL = total length of fish in cm.

Statistical Analysis

A preliminary analysis of the homogeneity and normality of the data was checked using Kolmogorov-Smirnov tests and Levene test. Once the normality was rejected, the data were statistically analysed by the Wilcoxon signed-rank test (nonparametric t-test) to reveal significant differences in means of reproductive variables (condition factors and fecundity) between two locations. One-way ANOVA followed by Tukey's post hoc test was used to resolve the significance differences in the GSI among various months. Average value of GSI of the fish of two ecosystems was compared using t-test. The results of sex proportions were tested using the chisquare test. Data are presented as means ± SD. All data were analyzed using SPSS (version 16.0). An error of probability level of less than 5% (P < 0.05) and a confidence of 95% are considered as the fiducial level of significance.

Results

Sex Ratio

In the river Ganga, the overall male to female ratio (σ : \mathfrak{P}) of *S. phasa* was 1:2.29 which was significantly different from the expected ratio (σ : \mathfrak{P} =1:1) of chi-square test (χ^2 = 4.159; df = 1; P < 0.05). Females outnumbered the males in the riverine population of *S.*

phasa throughout the year except for February and October. The details are given in Table 1. In the Hooghly

Table 1. Monthly sex ratio of males and females of S. phasa collected from river Ganga and H	Hooghly estuary.
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	River Ganga			Hooghly estuary				
Months	Abundance		Sex ratio	χ ²	Abundance		Sex ratio	χ ²
	്	Ŷ	(ơ:Չ)		ď	Ŷ	(♂:♀)	
Jan	39	39	1:1.00	0.000	37	39	1:1.28	0.254
Feb	41	38	1:0.73	0.485	44	41	1:0.79	3.365
Mar	43	54	1:1.85	3.587	46	51	1:1.31	0.688
Apr	46	61	1:1.94	5.330*	46	53	1:4.44	1.298
May	32	43	1:6.52	17.452*	42	39	1:0.75	0.438
Jun	36	48	1:3.00	8.000*	40	44	1:1.40	0.686
Jul	34	41	1:2.75	4.176*	33	37	1:2.33	1.905
Aug	37	46	1:2.29	4.159*	39	43	1:1.44	0.752
Sep	38	44	1:1.75	1.768	42	46	1:1.33	0.583
Oct	44	43	1:0.93	0.037	50	57	1:1.35	1.066
Nov	34	38	1:2.00	1.500	48	52	1.1.22	0.404
Dec	42	62	1:2.66	11.458*	44	56	1:1.86	3.956*
Total	466	557	1:2.29	4.159*	511	558	1:1.38	1.071

 σ =males, φ =females, χ^2 = chi-square value

*Significant values at the 5% level

estuary, the overall male to female ratio (σ : Q=1:1.38) was not significantly different from the hypothetical ratio of σ : Q=1:1 except during December as revealed by chi-square test (χ^2 = 1.071; df = 1; P > 0.05; Table 1). The percentage of females was high in Hooghly estuary during all the months except for February and May.

Maturity and Gonadosomatic Index

The maturity stages of gonadal development can be distinguished as: immature, developing, spawning capable, regressing and regenerating (Table 2). The details of the occurrence of the various maturity stages of the fish of both the ecosystems in different months are indicated in Figure 2. The spawning period of S. phasa ranged only once in a year from February to April in river Ganga, and high values of GSI were also synchronized during this period in both the sexes, which indicated the full development of their gonads (stage III). But in the same fish, the spawning was observed twice in Hooghly estuary where the ripe gonad (stage III) and high values of GSI were recorded during the periods between April-June and September-November (Figure 3). Overall, the average value of GSI in both the sexes was higher during the breeding season in both ecosystems, suggested peak spawning time of *S. phasa*. Monthly GSI values of both males and females of both ecosystems were significantly different (ANOVA, Tukey's post hoc test, P < 0.05) except during November-December in both the sexes of river Ganga (ANOVA, Tukey's post hoc test, P > 0.05). The average value of GSI of the fish of two ecosystems was significantly different (t-test, P < 0.01).

Oocyte Diameter

The frequency distribution of ova diameter measurements from a ripe ovary of *S. phasa* collected from Hooghly estuary showed that it was multimodal type having three groups of ova (immature, maturing

and mature) which indicated that the fish spawns more than once in a year. The size of ova ranged between 0.30-1.19 mm and fully matured and ripe eggs (diameter 0.80-1.19 mm) were recorded during May and November in Hooghly estuary. However, *S. phasa* procured from Ganga River was unimodal type (one type of egg) and the size of ova ranged between 0.26-1.05 mm. Fully matured and ripe eggs (diameters 0.76-1.05 mm) were recorded during March in river Ganga (Figure 4).

Fecundity and Condition Factor

The absolute fecundity in the specimens of *S. phasa* of the river Ganga varied between 3,600-24,000 with an average value of 10254±0.34 while it ranged between 3,100-20,100 in the fish of estuary with an average value being 10111±0.57 which was found to be significantly different (P < 0.001) as revealed by Wilcoxon signed-rank test (nonparametric t-test) between the two sampling sites. The recorded average condition factor of the combined male and female ranged between 0.42-0.51 and 0.62-0.81 with the average value being 0.47±0.02 and 0.89±0.63 in *S. phasa* of the river Ganga and Hooghly estuary respectively, which was significantly different (P < 0.001) as depicted by Wilcoxon signed-rank test (Figure 3).

Relative fecundities showed linear and positive correlation ($R^2 > 0.90$) for TL, BW, OL and OW of both the ecosystems, which were found to be significantly different (P < 0.001) for TL, BW and OW while non-significant for OL between the populations (Table 3).

Discussion

Sex ratio determinates the reproductive potential of a fish population that aid in the assessment of the reproductive biomass and fecundity of the total population (Marshall *et al.*, 1998, 2006). In the present study, the biased sex ratio was observed where females

Stage	Ovaries	Testes
l (Immature)	Ovaries small, thin and white in colour. Eggs very minute	Testes small, slightly elongated, distinguished
	and distinct only under microscope.	microscopically Vasdeference thin
II (Developing)	Ovaries yellow in colour, granular in consistency, ova	Testes elongate, white and opaque.
	visible to the naked eye. Oviduct reduced.	Vasdeferentia wide but reduced.
III (Spawning	Ovaries yellow in colour elongated occupying whole-body	Testes creamy white or reddish white in colour
capable)	cavity. Blood vessels visible over the surface of the ovaries.	occupying more than half of the body cavity.
	Ova extruded on light pressure, eggs were opaque and distinct.	Viscous fluid oozes out from cut ends.
IV (Regressing)	Ovaries yellow, enclosing the intestine by lateral extension. Oviduct reduced.	Testes elongated reddish or creamy white in
		colour, Vasdeferens not discernible.
V (Regenerating)	Ovaries flaccid contracted and usually empty. The ovary	Testes white, weight reduced. No milting on
	has few remnants of mature ova in recent spawned fish.	pressure.
	Weight reduced.	

Table 2. Criteria used for the determination of maturity stages of S. phasa collected from river Ganga and Hooghly estuary
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Figure 2. Maturity stages of *S. phasa* in different months collected from river Ganga ((A) males and (B) females) and Hooghly estuary ((C) males and (D) females).

dominated the males throughout the year in the riverine population of S. phasa except for February and October, while in Hooghly estuary; the overall male to female sex ratio was not significantly different from the hypothetical sex ratio except during December. Tsikliras and Koutrakis (2013) reported the female-biased population in the Sardina pilchardus and pointed out that the unequal sex ratio is natural in fish populations. Several researchers (Kabir et al., 1998; Corrêa et al., 2005; Deshmukh et al., 2010; Roomiani et al., 2014; Lopes et al., 2018) also reported the occurrence of female dominancy over male in various species of Clupeiform under different environmental conditions. Tsikliras et al. (2010) reported a female-biased sex ratio in 72% of the fish species due to an adaptive strategy to boost the egg numbers to promote the recruitment of more offspring (García-Abad et al., 1998). The female biased population of the fish could be due to the differentiation in growth, longevity and mortality rate between the sexes or the energy costs of reproduction (Potts & Wootton, 1984; Marshall et al., 1998; Vicentini & Araujo, 2003; Zhang et al., 2009; Mahmood et al., 2011). The biased sex ratio may be because of ecological factors (Gamble & Zarkower, 2012), abundancy of forage items or reproductive behaviour (Marshall et al., 1998; Vicentini & Arau'jo, 2003; Mahmood et al., 2011; Vandeputte et al., 2012). Zhang et al. (2009) also pointed out the factors such as growth rate, sex reversal and migration for biased sex ratio in different species of the fish. However, in the present study, amphidromous and potamodromous migration may be the reason for the biased sex ratio in the fish population.



Figure 3. Mean ± SD of the gonadosomatic index (GSI) and condition factor (K) of males and females in *S. phasa* of river Ganga (A) and Hooghly estuary (B).



Figure 4. Size Frequency distribution of intraovarian oocytes of S. phasa collected from river Ganga (A) and Hooghly estuary (B).

Table 3 Regression analyses of relative fecundities in S. phasa collected from river Ganga and Hooghly estuary.

Ganga River		Hooghly Estuary			
Parameters	Regression equations	R ²	Regression equations	R ²	Р
TL vs F	Log F= 2.2182 + 0.8937 Log TL	0.9826	Log F=3.10 + 0.8010 Log TL	0.9740	0.0001*
BW vs F	Log F= 2.5493+ 0.8765Log BW	0.9639	Log F= 2.534+ 0.8321 Log BW	0.9621	0.0219*
OL vs F	Log F = 2.8067+1.9406Log OL	0.9652	Log F = 2.6032+1.356Log OL	0.9640	0.9264 ns
OW vs F	Log F=3.2161+1.2958Log OW	0.9730	Log F=3.12+0.456Log OW	0.9681	0.0057*

F=fecundity, TL=total length, BW= eviscerated body weight, OL=ovary length and OW=ovary weight, R² = correlation coefficient

Note. ns: not significant

P =*Significant values at the 5% level

The large and healthy individuals of female fish are less vulnerable for predation and generate maximum number of high-quality gametes because of more energy stores in their muscle and liver for the growth and development of their gametes (Helfman *et al.*, 1987; Jonsson & Jonsson, 2015). The occurrence of larger and heavier females of *S. phasa* in the present study was found to be in corroboration with other clupeids (Kabir *et al.*, 1998; Lopes *et al.*, 2018), although the sex ratio at size varies temporally and spatially according to Arocha and Barrios (2009).

Five maturity stages were recorded during the present study after following the scheme of classification given by Brown-Peterson *et al.* (2011). But,

Jhingran (1961) recognized seven stages of maturity in *S. phasa* after following the scheme as given by Hjort (1911). In the specimens of the fish of Ganga River, the high value of GSI was recorded in both sexes during the period from February to April because of the full development of gonads. Recovering gonads remained quiescent from September through January in the fish of river Ganga. An increase in the development of gonads took place during February/March, followed thereafter, by rapid changes in gonads. The peak condition of the development of gonads in the fish of river was recorded during March. However, in Hooghly estuary, two peaks were observed in a year in both the sexes of *S. phasa* which coincided with the rainy and post rainy seasons.

In the specimens of Hooghly estuary, high GSI in both sexes were observed during May and November which represents full development of gonads during these months. However, it is relevant to mention that according to Mookerjee and Mookerjee (1950) clupeids breed in the tidal areas during the periods from January through July, while Jones and Menon (1952) reported the peak breeding period of S. phasa during October-November on the basis of the collected developing eggs, larvae and juveniles from Hooghly estuary near Barrackpore. According to the present study, S. phasa breeds more than once in a year in Hooghly estuary and once in river Ganga at Allahabad-Varanasi region. A survey of literature showed that, in tropical and subtropical regions, clupeids reproduce throughout the year (Wootton & Smith, 2015). Kabir et al. (1998) and Almukhtar et al. (2016) also recorded the two spawning peaks in Gadusia chapra and Tenualosa ilisha respectively. The breeding process has a direct relation with the onset and duration of monsoon and flooding cycles (Sarkar et al., 2010), perhaps this is the reason why the breeding season in most fishes is not the same in different regions of the country. In the present study, the sampling sites of S. phasa are located in tropical region where well-defined seasonal variations do occur, and the difference in the spawning period might be related to variations in the environmental conditions. A lot of notable researchers like Sarkar et al. (2018, 2019), Karnatak et al. (2018, 2020) established the correlation between GSI and climate variables and emphasized that climatic variability particularly temperature and seasonal rainfall was considered to be important for the regulation of the reproductive cycles in the teleostean fish which may lead the region-specific changes in breeding phenology.

The GSI values and condition factor indicated no direct relation in S. phasa of both the ecosystems. Similar to this study, Cardoso et al. (2019) reported the opposite pattern for mean condition factor (K) and GSI. The high absolute fecundity was recorded in S. phasa of Ganga River as compared to those living in estuary. Guèye et al. (2012) pointed out that the efficiency of foraging of fish species and prey availability influence the condition and reproductive potential in fish. Variation in the number of eggs produced by the individual fish is generally dependent on the factors like overexploitation, habitat degradation, unsustainable harvesting, food availability, size, age and condition of the particular species (Bhuiyan et al., 2006; Sarkar et al., 2012b; Gupta et al., 2014). Several researchers (Sarkar et al., 2009, 2012b) reported that factors such as time of sampling, maturation stage and nutritional status affect the fecundity between fish populations.

In the present study, fish collected from estuary have low absolute fecundity and high ova diameter while the fish of river Ganga showed high absolute fecundity and low ova diameter. Several researchers pointed out that the intraspecific discrepancy in mean egg dimension may likely owing to variation in intimacy and population density of spawner or due to specific ovulation time and the stage of egg development (Ezenwa, 1981) and could also because of water turbulence in defined ecosystems (Denny & Shibata, 1989; Levitan & Petersen, 1995). Smith and Fretwell (1974) and Einum and Fleming (2004) documented that mean increase in egg size and decrease in egg number may occur because of environmental variability and its quality. Lahti and Muje (1991) reported that eggs of large size with a relatively small number are an adaptation to the poor food supply for the juveniles. The variations in relation between fecundity and ova diameter were due to the differences in individual ovulation time, stage of egg development and abiotic factors (Ezenwa, 1981; De Silva, 1986; Legendre & Ecoutin, 1989). Guèye et al. (2012) also established the trade-off between fecundity and egg size in fish.

Significant variations were recorded in the reproductive traits (fecundity, oocyte diameter, condition factor, GSI) between two populations of S. phasa collected from two different ecosystems viz. freshwater and estuarine. The present study corroborated with the earlier study of Gueye et al. (2012) who reported the significant variations in the reproductive traits (fecundity, oocyte diameter, oocyte weight, condition factor, GSI) of females black chinned tilapia collected from three different ecosystems (coastal marine, freshwater and estuarine). A lot of previous studies (Gueye et al., 2012; Sarkar et al., 2017; Praveen et al., 2017) have also reported the intraspecific differences in reproductive traits (fecundity, oocyte diameter, condition factor, GSI) between the populations of the fish and emphasized that these variations reflect the adaptation of fish to the local environmental conditions (Taylor, 1991; Conover & Schultz, 1997; Sarkar et al., 2017; Praveen et al., 2017).

A linear relationship was found to exist between fecundity and each one of these four parameters (TL, BW and OW and OL). The fecundity was better correlated with TL followed by BW, OW and OL. Similar, results were reported by other researchers (Sarkar *et al.*, 2009; Mir *et al.*, 2013; Gupta *et al.*, 2014; Kashyap *et al.*, 2016; Faridi *et al.*, 2020) in different fish species.

Conclusion

The intraspecific variations in reproductive traits reflect the environmentally induced reproductive plasticity in the fish. The study of the reproductive cycle and fecundity is essential and fundamental for understanding the fish population dynamics to obtain a sustainable harvesting. The baseline data on the reproductive biology of little known species are helpful for the development of stock assessment models, which can be utilized further for effectively scientific sustainable management of natural population of the overexploited fish or degraded habitats. Moreover, the knowledge of reproductive biology of the fish is considered to be important and helpful for adequate implementation of management measures in restoring the depleted stocks of the fish species because ambiguity in species biology could hinder accurate stock assessment and monitoring.

Ethical Statement

This study does not need any formal consent as the experimental fish, *Setipinna phasa* is widely distributed in the river Ganga at Allahabad-Varanasi region and Hooghly estuary. The collection sites were not restricted areas but fishing is carried out commercially. The species is designated as 'least concern' by IUCN's Red List of threatened species. The study complies with the existing rules and guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), guidelines for laboratory animal facility, Government of India.

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