

# Larval Development of *Limnoperna fortunei*

Daniel H. Cataldo

**Abstract** Mature sperm cells of *Limnoperna fortunei* measure about 4  $\mu\text{m}$ , and ova are typically spherical, 80–100  $\mu\text{m}$  in diameter. Forty minutes after spawning, the first polar lobe appears, and the first division occurs 14 min later. Slightly over an hour after spawning, the second polar lobe appears and the second division yields a 4-cell stage. The third division occurs 90 min after spawning, and the fourth 115 min after spawning. Approximately 3.5 h after spawning (at 26 °C) the morula stage is reached. Six hours after spawning, the first trochophores appear (95–110  $\mu\text{m}$  in length) at 28 °C. Subsequently, the prodissoconch I starts developing, initially as small rosette-shaped structures on the dorsal side of the trochophore. Straight-hinged veligers (115–160  $\mu\text{m}$ ) start appearing 24 h after spawning. These larvae start feeding externally and secrete the prodissoconch II. Umboned veligers (156–220  $\mu\text{m}$ ) are reached 287 (at 28 °C), 165 (25 °C) and 118 (20 °C) h after spawning. From there on, the larva reabsorbs its velum and develops a muscular, adhesive foot, yielding a plantigrade larva (250–405  $\mu\text{m}$ ), which shortly thereafter settles and attaches to the substrate. Development times are therefore strongly influenced by water temperature.

**Keywords** *Limnoperna fortunei* · Larvae · Veliger · Development · Morphology

## Introduction

Freshwater bivalves have evolved different strategies to maximize survival and dispersion. While most marine species have free-living larvae, many freshwater species have a unique ectoparasitic larval stage, the glochidium, which attaches

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to the gills of fish. During early stages of development, these larvae are incubated in marsupia in the adults. Once these stages have developed into the glochidial stage, they are released into the water where they need to quickly reach a host fish in order to develop into a juvenile. While this strategy necessitates finding the right fish to complete development, it insures that larvae are not washed out to sea. Other freshwater species incubate their offspring to avoid being lost to unfavourable downstream environments, and larval development is completed in specialized gill pouches in the adult. Once the miniature adult is large enough, it falls directly to the bottom (e.g. Corbiculidae). In this brooding strategy, the risk of expatriation is decreased, but dispersal opportunities are sacrificed.

In contrast, *Limnoperna fortunei* develops through a series of planktonic larval stages. This has the benefit of effective and rapid downstream dispersal and the ability to conquer new environments, but it risks expatriation of larvae to areas unsuitable for survival, including estuaries and the sea. Evolution suggests that for the vast majority of freshwater organisms, in the balance between enhanced dispersion and risk of expatriation into hostile environments, drawbacks from expatriation are more important than advantages of dispersion, and therefore free-swimming larvae of marine ancestors have been mostly lost. Nevertheless, *L. fortunei* seems to have benefited from having retained a free-swimming larval stage (see Chapter “Distribution and Colonization of *Limnoperna fortunei*: Special Traits of an Odd Mussel” in this volume).

Development of *L. fortunei* is typical of planktotrophic larvae, and characterizes many mytilids and dreissenids (including the zebra mussel *Dreissena polymorpha*). The initial trochophore stage is followed by a veliger and, subsequently, by D-shaped larval stages (at which time prodissoconch 1 and 2 are secreted), followed by the newly settled juvenile (the dissoconch stage) (Ockelmann 1995). Organisms, such as *L. fortunei* that utilize a planktotrophic larval development strategy typically start as small eggs and require a long larval development period. These larvae feed on plankton and their development is prolonged compared to species with lecithotrophic larvae. This favours long distance dispersion of planktotrophic species both in their natural environment and through the ballast water of ships on the high seas.

## Methodological Approaches

Description of mollusc larval stages can be carried out (1) on the basis of field-collected materials, (2) on the basis of larvae obtained in the laboratory inducing spawning of ripe adults and following fertilization and development under controlled conditions or (3) a combination of the above, tracking the development of field-collected larvae in the laboratory.

The first descriptions of the larval stages of the golden mussel were undertaken on larval specimens collected with plankton nets and whose development was subsequently followed in the laboratory (Choi and Kim 1985; Choi and Shin 1985). Santos et al. (2005) analyzed larval stages collected bimonthly in Guaíba Lake in

Rio Grande do Sul, Brazil. Ezcurra de Drago et al. (2006) also described larval development stages from field-collected samples undertaking studies in 1997–2000 at various sites along the Middle Paraná River (31°38'S, 60°40'W). While field-collected samples allow for the adequate description of the morphology of each developmental stage, they are not appropriate for determining the time required for each stage to transition to the next.

Cataldo et al. (2005) analyzed the embryonic development of *L. fortunei* by inducing adult specimens to spawn in the laboratory and identifying the various larval stages and tracking the time needed to reach each developmental stage. In their study, Cataldo et al. (2005) used three experimental temperatures, 20, 25 and 28 °C, which generally span the temperatures during the reproductive period of the mussels in South America.

The majority of experimental studies on bivalves have used serotonin as the agent to induce the production of gametes (Matsutani and Nomura 1982; Braley 1985; Ram and Nichols 1993; Vanderploeg et al. 1996); however, serotonin does not produce satisfactory results in *L. fortunei*. Cataldo et al. (2005) used an antifouling molluscicide commercially known as Spectrus CT1300 (n-alkyl dimethylbenzyl ammonium chloride, a quaternary ammonium) (Cataldo et al. 2003; see Chapter “Chemical Strategies for Control of the Golden Mussel (*Limnoperna fortunei*) in Industrial Facilities” in this volume), which proved to be very effective at concentrations of 0.75 ppm (active ingredient), yielding ripe gametes in over 90 % of the experimental beakers 30–180 min after exposure of the adults to the chemical. In the remaining 10 % of mussels, it took up to 8 h for gametes to appear (Cataldo et al. 2005).

Analysis of developmental times for the larval stages of this species, in particular the time it takes for larvae to reach settlement stage, has both theoretical and applied interest. Learning more about the basic biology of *L. fortunei* allows for a better understanding of the mechanisms governing the dispersion of this species and also allows for comparisons with other molluscs in order to highlight common behavioural patterns based on shared constraints. For the purposes of controlling this pest in industrial installations, it is potentially useful to know the origin of the populations that are actually seeding the individuals fouling pipes and intakes, and knowledge of the duration of each developmental stage at different temperatures has important implications for developing treatment methods. Knowledge of these larvae is also necessary for early detection and monitoring of colonization by *L. fortunei*.

## Developmental Stages

Larval development of *L. fortunei* can be divided into two main stages. The first stage comprises nonshelled development from fertilization until the formation of the trochophore larva. The second stage is characterized by shelled forms from veliger to plantigrade larva, at which point the animal is capable of binding to the substrate.

## ***Gametes and Nonshelled Developmental Stages***

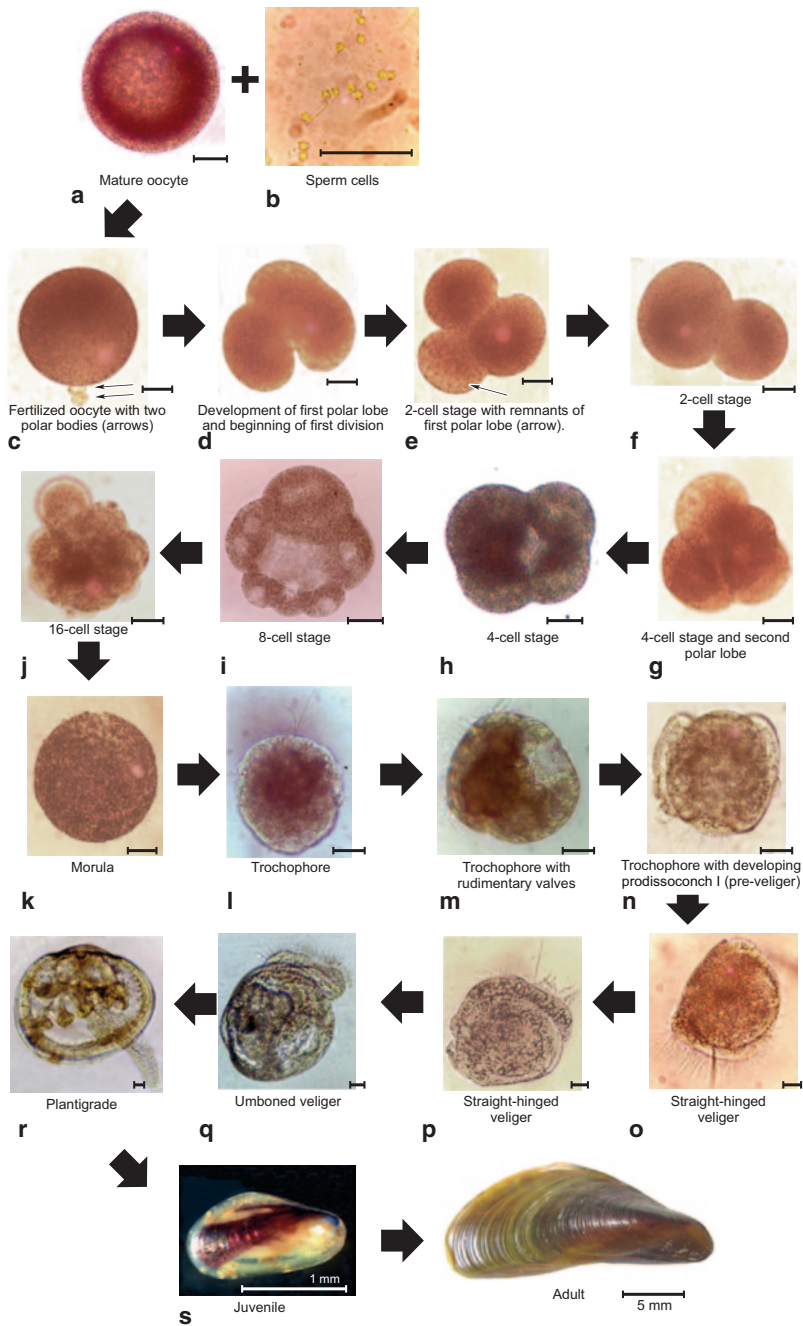
Mature sperm cells measure about 4  $\mu\text{m}$  (excluding the tail) and are highly mobile (Fig. 1b). Ova, of typically spherical form, are 80–100  $\mu\text{m}$  in diameter (Choi and Shin 1985; Cataldo et al. 2005) (Fig. 1a). These cells are still diploid upon release and chromatic reduction takes place in the medium by the production of polar bodies (Fig. 1c).

Segmentation is similar to that observed in many invertebrates and in most molluscs, and complete spiral cleavage yields a characteristic trochophore larva. Segmentation starts about 40 min after fertilization, producing the first polar lobe, and very shortly thereafter, the first cellular division takes place (Fig. 1d,e and f). After a few minutes, the first polar lobe is resorbed by one of the daughter cells (Fig. 1f). At 26°C, this first division, yielding two uneven blastomeres, occurs 54 min after fertilization. Approximately 11 min later (slightly over 1 h after spawning), the second polar lobe appears (Fig. 1g) and the second division starts, yielding a 4-cell stage (Fig. 1h). The third division takes place along the equatorial plane, separating four micromeres from four macromeres; at 26°C, this 8-celled stage occurs 90 min after fertilization (Fig. 1i). Twenty-five minutes later (115 min after fertilization), the fourth division yields the 16-celled stage (Fig. 1j). Approximately 3.5 h after fertilization (at 26°C) the morula stage is reached (Fig. 1k).

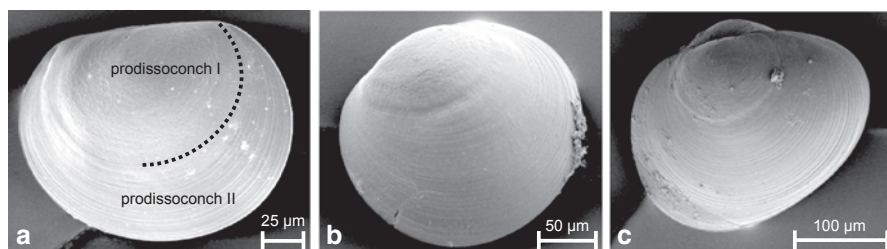
The morula is ciliated and has limited, poorly coordinated movement, only occasionally leaving the bottom and venturing into the water column. Six hours and twenty minute after fertilization, the first trochophores (95–110  $\mu\text{m}$  in length) appear (Fig. 1l). These active larvae have a well-developed apical tuft of cilia that allows for well-coordinated swimming. Most of these larvae wander freely in the water column and rarely rest on the bottom. Shells appear as small rosette-shaped structures on the dorsal side of the trochophore (Fig. 1m). The first shell, prodissococonch I, is secreted by the shell gland, and begins to split into two, slowly coating the soft tissues of the larva. The nutrition of the larva is supplied exclusively from yolk reserves until the trochophore stage. The transition period between the trochophore larva and the next stage—the veliger larva—is also referred to by some authors as a pre-veliger larva with incomplete shells (Fig. 1n; Ezcurra de Drago et al. 2006).

## ***Shelled Developmental Stages***

The first shelled stage is termed the veliger larva, D larva, or straight-hinged larva (since the dorsal margin of the shell is straight). This stage starts when the valves completely cover the body of the animal. This larva has a fully developed velum located anteriorly and is provided with a strip of cilia and a central group of flagella (Ezcurra de Drago et al. 2006; Fig. 1o and p). Once the velum has completely developed, the larva begins to feed on plankton. From this phase onwards, the shell is secreted by the mantle (rather than by the larval shell gland), and the prodissococonch II is formed. This transition is marked by the appearance of growth lines, which define



**Fig. 1** Larval stages of *L. fortunei* (scale bars are 25  $\mu$ m, unless otherwise noted). (Modified from Cataldo et al. 2005)



**Fig. 2** SEM photographs of the shell of a straight-hinged larva (a), umboned larva (b) and plantigrade larva (c). (Adapted from Cataldo et al. 2005)

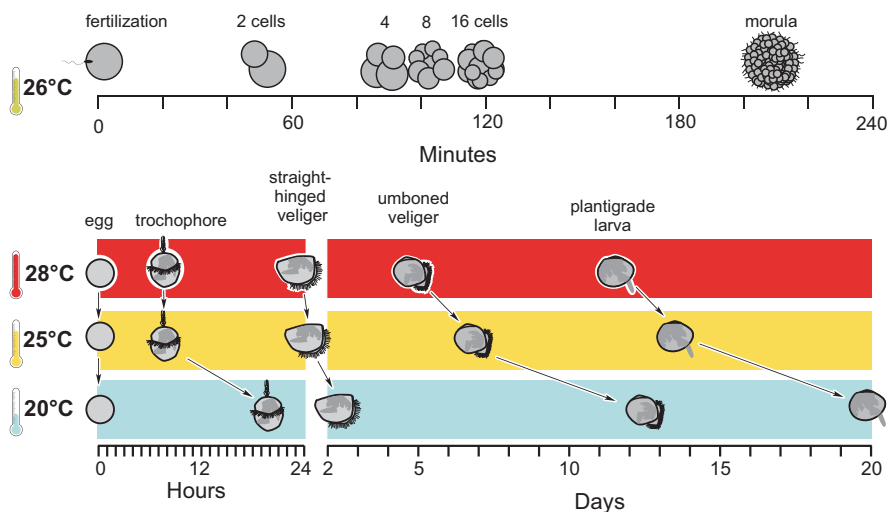
the start of the prodissoconch II (Fig. 2a). Straight-hinged larvae are 115–180 µm in length (Choi and Kim 1985; Cataldo et al. 2005; Santos et al. 2005).

The dorsal margin of the shell, which is initially straight, gradually bulges and the umbo appears as a progressively more conspicuous bump, giving origin to umboned veligers. These larvae are 190–230 mm in length (Choi and Kim 1985; Cataldo et al. 2005; Santos et al. 2005; Fig. 1q and 2b). During this stage, the prodissoconch II completes its growth, whereas the prodissoconch I remains restricted to the dorsal margin and forms part of each umbo (Fig. 2b).

As larvae develop, swimming activity becomes progressively slower and the animals tend to spend more time on the bottom of the vessel. Shortly before settling, each larva reabsorbs its velum and develops a muscular, adhesive foot, giving rise to a plantigrade larva (around 250–405 µm in length; Choi and Kim 1985; Cataldo et al. 2005; Santos et al. 2005; Fig. 1rr and 2c). At this stage, the only means of locomotion is the foot and the valves begin to elongate. The organism completes its internal development and the siphons and gills are easily observed. This stage is associated with exploratory behaviour of the substrate and concludes with the attachment of the byssal threads.

## Effects of Temperature on the Larval Development of *L. fortunei*

The effect of temperature on the rate of larval development was analyzed by Cataldo et al. (2005) through induced spawning, fertilization of gametes and incubation of eggs under controlled laboratory conditions at three temperatures (20, 25 and 28 °C). As expected, the fastest developmental rates were observed at the highest temperature (28 °C), and decreasing at 25 °C and further at 20 °C (Fig. 3, lower panel). Differences in developmental times for all stages surveyed were almost twice as high between 20 and 25 °C as they were between 25 and 28 °C (Fig. 3). Size, on the other hand, varied little with temperature (Cataldo et al. 2005).



**Fig. 3** Developmental times of *L. fortunei* at 26°C from fertilization to morula (*upper panel*), and at three different temperatures to plantigrade larva (*lower panel*) (figures are not to scale). (Based on data from Cataldo et al. 2005)

A 20°C (which is 3–5°C above the lower thermal limit for reproduction of the species in the lower Paraná River delta; see Chapter “Reproductive Output and Seasonality of *Limnoperna fortunei*” in this volume; Cataldo and Boltovskoy 2000), the trochophore larval stage is reached ~20 h after fertilization. The stage of straight-hinged veliger appears after 45 h. The umboned veliger larva develops after 11 days and is actively swimming. Swimming behaviour slowly becomes less vigorous, and larvae tend to descend towards the bottom transforming from a swimming to a crawling phase. Beginning at day 20, the foot develops, and the plantigrade larval stage is reached (Fig. 3).

At 25°C, the growth rate is significantly higher than that observed at 20°C. Trochophore larvae develop within 6–7 h after fertilization, while the ‘D’ form (D-shaped or straight-hinged) appears 26 h after fertilization. From the 4th day, the hinge bends leading to the umboned stage, and the foot is developed beginning on the 13th day, about 7 days sooner than at 20°C (Fig. 3).

A 28°C (which are typically the highest summer water temperatures in the lower delta of the Paraná River and the Río de la Plata estuary), development is about twice as fast as at 20°C, and approximately 20% faster than at 25°C. At 6 h, the trochophore larva develops, and the straight-hinged veliger stage appears just after the 1st day. The umbo is evident beginning on the 4th day, which is 2 days earlier than at 25°C, and a full week before those incubated at 20°C. On day 11, organisms have a functional foot, and they are exploring the substrate by crawling on the bottom and walls of the chamber.



The information reported by Cataldo et al. (2005) for 28 °C overlaps the developmental times in Choi and Kim (1985) for the straight-hinged stage (23 h) (see Fig. 3). However, the times reported by Choi and Kim (1985) to reach the unbanded larval stage (10 days) and the pediveliger stage (18 days) are considerably longer than those reported by Cataldo et al. (2005) (~5 and 11 days, respectively). The study by Choi and Kim (1985) used larvae that were collected from the plankton, and hence the elapsed times following fertilization were not exactly known. Similarly, the water temperatures of their laboratory experiments were not reported. The laboratory study of Cataldo et al. (2005) clearly showed that the velum is fully developed by the time the dorsal side of the animal shows a straight hinge between the two valves, however in Choi and Kim's (1985) study, early D-shaped larvae were still devoid of a velum; they only observed a velum in middle D-shaped larvae. This discrepancy may be due to artefacts induced through plankton sampling, whereby large proportions of net-sampled larvae are stressed or dead, and they show little or no mobility since their vela are retracted and inconspicuous.

Comparison of the developmental rates of *L. fortunei* with those reported for the zebra mussel, *D. polymorpha*, indicates that both are roughly similar. The zebra mussel reaches the D-shaped stage in about 30–70 h (Sprung 1987; Nichols 1993; Stoeckel et al. 1996), whereas the golden mussel reaches this stage between 24 and 50 h (Cataldo et al. 2005). Vanderploeg et al. (1996) reported that settlement of *D. polymorpha* occurs at 15–22 days, and the plantigrade larval stage of *L. fortunei* appears on days 11–20 (Cataldo et al. 2005). The developmental rates reported for some marine mytilids are within the ranges found for *L. fortunei* (e.g. *Perna viridis*, Tan 1975; Siddall 1980; *Mytilus platensis*, Penchaszadeh 1980; *Modiolus modiolus*, Schweinitzd and Lutz 1976), whereas they are significantly slower for other marine mytilids. For example, it can take *Mytilus edulis* up to 35 days to reach the veliger stage, and it may take over 6 months for it to complete metamorphosis (Bayne 1976). These comparisons are not well refined, since the modulating effects of temperature have not always been accounted for in these studies, but most marine bivalves with free-swimming larvae seem to have slower development rates. Over 80% of the 37 marine bivalves surveyed by Thorson (1961) have development times longer than those of *L. fortunei* (at 28 °C), and only 5% have faster rates. Accelerated development may be an adaptation to colonize freshwater environments. Indeed, whereas marine benthic organisms may gain significant advantage from extended larval periods (e.g. Scheltema 1986), many freshwater animals can incur the danger of being flushed out to the ocean unless settling occurs more-or-less rapidly. Estuarine larvae use vertical migration to overcome seaward transport (e.g. Cronin 1982), but this is not feasible in the turbulent conditions of streams and rivers. Furthermore, the near-bottom high-saline waters involved in estuarine upstream water transport (Guerrero et al. 1997; Acha et al. 2008), are unfit for the survival of *L. fortunei* (Sylvester et al. 2013). Thus, it is conceivable that the relatively short development times of *L. fortunei* have evolved to overcome the expatriation hazards associated with planktonic larvae.



## Mortality Rates

In their laboratory study of larval development, Cataldo et al. (2005) noticed very high mortality rates for *L. fortunei*, around 80–90%. However, mortality was not even throughout development, and it was highest during the transition from the straight-hinged to the umboned veliger stage. High mortality rates during the transition between these two stages of development have also been reported for other bivalves (Wada 1968).

By tracking cohorts of zebra mussel during their downstream drift in the Illinois River (USA) and through laboratory rearing experiments, Schneider et al. (2003) concluded that the mortality of the planktonic larvae of *D. polymorpha* was significantly higher during the transition from straight-hinged to umboned veligers, than during any stage before or after this period. Larval mortality can respond to many different stressors, including scarce or inadequate food supply, pollution, predation, advective sinking in the water column, etc. (Morgan 1995). However, most of these stressors should affect all larval stages similarly. This suggests that peak mortalities are associated with the major changes in larval morphology and anatomical reorganizations, including the formation of a digestive tract and accompanying peaks in metabolic activity, that take place during the transition from the straight-hinged to umboned stage (Sprung and Widdows 1986; Fujimura et al. 1995; Schneider et al. 2003). These shifts may cause physiological or alimentary stress and result in higher mortality (Schneider et al. 2003).

## Concluding Remarks

Industrial plants and water treatment facilities become infested with *L. fortunei* when free-swimming larvae pass through protecting grids and filters of the intake pipes. Afterwards, larvae settle out onto these same grids and filters as well as pipes, heat exchangers and other surfaces, and cause severe fouling problems (see Chapter “Impacts of *Limnoperna fortunei* on Man-Made Structures and Control Strategies: General Overview” in this volume). Since residence times of water are low (usually <1 h) in cooling systems, only very late plantigrade larvae are retained within plant components, whereas earlier stages are released with the effluent water. The development times reported by Cataldo et al. (2005) indicate that the dense populations usually present on the outer and inner hard surfaces of the plants have no impact with regards to further infestation of the plant’s inner components. Fouling populations could seed internal surfaces only if water flow regimes within or around plant intakes are highly irregular and abundant ‘dead-water sites’ with very high water residence times are present. On the other hand, it is possible to estimate the location of seeding populations by using these development times and taking into account water temperature and flow characteristics. For example, since both the Paraná and the Uruguay rivers have mean

flow speeds of about 0.3 m/s, the seeding populations for the biofouling-affected plants located in Buenos Aires along the Río de la Plata estuary are ~250 km upstream in the summer, when water temperatures are above 25 °C, and ~500 km upstream in the autumn and spring, when water temperatures are below 20 °C. Thus, fine-tuning our knowledge of temperature-dependent development times can contribute to the assessment of the fouling mechanisms and help to develop sound control measures.

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